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INHERITANCE OF THE ALBINISTIC NON-CONIDIAL CHARACTERS IN INTER-SPECIFIC HYBRIDS IN NEUROSPORA*

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(WITH PLATES 1–7)

When an albinistic race of *Neurospora sitophila* is crossed with a conidial race of the species (PLATE 1), four of the spores from an F_1 ascus will give rise to albinistic mycelia and the other four will develop mycelia with typical monilioid conidia (PLATE 2). If these eight mycelia, which are the progeny of a single zygote "mother-cell," are mated in all possible combinations, four of the cultures will give rise to perithecia which produce only albinistic ascospores, eight cultures will mature asci heterozygous for conidia, and four cultures will produce only typical ascospores whose mycelia will bear monilioid conidia, illustrating Mendelian segregation in a very striking manner (**3**). Continuing from this point, the present paper records the results of experiments in crossing an albinistic race of *N. sitophila*, not only with a race morphologically indistinguishable from *N. tetrasperma*, which has four-spored asci, but also with *N. crassa*, another species with eight-spored asci. In the course of this work certain hermaphroditic hybrid segregate strains were developed which were

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more or less self-incompatible, but which crossed freely with the unisexual tester strain. Other fruit bodies which appear to be hybrids between two strains, each of which was fully capable of producing ascocarps by itself, were also obtained illustrating unique cases of a preference for cross-fertilization over selfing. These races will also be described.

Previous contributions on the morphology and cytology of species of Neurospora and interspecific hybrids are referred to in the paper cited above, and they need not be reviewed again, except to point out that when typical *sitophila* was crossed with typical *tetrasperma* (**8**) the segregations of factors for four-spored and eight-spored asci were certainly not according to any simple Mendelian formula. Allen (**1, 2**) crossed non-tufted races of *Sphaerocarpos Donnellii* with tufted races, and tufted with tufted races, and showed that segregations for the tufted character are not as clear cut as would be expected in simple Mendelian inheritance.

Since one of the parents used in making the interspecific crosses to be described was an albinistic race of *sitophila*, a race which produces a fluffy aërial growth and which seems to be sterile as to production of monilioid conidia, it would not be unexpected to find the results still further complicated if, instead of selecting a typical conidial strain of *N. tetrasperma* as the other parent, a four-spored segregate, no. 507, which produces only a scanty aërial growth with only a few conidia were chosen. When this was done, the results turned out to be even more irregular, conflicting and difficult to explain than was expected. On the other hand, crosses between albinistic non-conidial *sitophila* and typical conidial *crassa*, being crosses between species both of which have eight-spored asci, illustrate again rather simple Mendelian segregation. Only a comparatively few matings were studied and they will be described first, although the results obtained by crossing albinistic *sitophila* with the four-spored segregate no. 507 should be of far greater interest from a genetic standpoint.

Neurospora crassa is crossed with *N. tetrasperma* with great difficulty, and f_1 ascospores are seldom matured in cultures of such matings. Some strains are more fertile when mated than

others, so that by mating several different sets of ascosporic strains one may be certain of finding a combination that will eventually mature ascospores. The perithecia, asci and ascospores of *N. crassa* are much larger than are those structures of *N. sitophila*. Nevertheless, fertile hybrids between typical strains of these two species have also been produced on several occasions. Segregations of the factors for sex and for conidia are the only ones which will be considered in the crosses between albinistic *sitophila* and typical conidial *crassa*.

ALBINISTIC *Neurospora sitophila* × TYPICAL *N. crassa*

Matings were made in test tube cultures on corn meal agar between mycelium no. 42a, a typical sex A race of *N. crassa*, and no. 56(2 × 6)a.2, which is an albinistic sex B race of *N. sitophila* (**3,** p. 18) and one of the progeny from a mating of two albinistic races. Each of the cultures finally developed a few perithecia and after about six weeks some ascospores were discharged. No extended observations were made as to the nature of the F_1 asci and their spores due to other interests at the time. Six months later, surface sowings of the f_1 ascospores were made in the usual way, and after heating the plates to kill any conidia present and to stimulate ascospore germination, it was found at the end of four hours that practically every one of the mature ascospores had germinated. Forty-four germinating spores which were in a position to be readily isolated were transferred to fresh agar plates where they were allowed to grow until the tip ends of the germ tubes were long enough to be cut off and transferred to tubes without including the original bit of agar bearing the ascospore itself and any possible conidia.

In this way forty-four cultures of single ascospore f_1 haplonts were obtained. At the end of seven days it was clear that three or four different types of mycelia were developing. Haplonts 11, 15, 20, 23, 30 and 41 were albinistic, with profuse, fluffy, whitish, aërial growth and no conidia. Mycelia 40 and 44 appeared to be albinistic, yet a few conidia which remained attached for a long time were formed. Mycelia 8, 27 and 33 tended to be albinistic, but developed a rather scanty growth of pale aërial hyphae with only a few conidia. Of the other thirty-three

mycelia, some developed conidia quickly and profusely and others slowly, but finally all resembled very much typical cultures of the *sitophila* parent. Just why only six out of the forty-four mycelia were strictly albinistic and sterile as to conidia, and five others were really intermediates, is not clear. The albinistic haplonts which produced no conidia were back-crossed with stock tester strains, sex A and sex B of the conidial parent, *N. crassa*. Mycelia 11, 20, 23, 30 and 41 proved to be of sex A, and no. 15 was of sex B class. The other haplonts were not tested as to their sex.

The test culture in which the f_1 albinistic haplont 20 was grown with typical *crassa*, sex B, matured ascospores first. These back-cross asci were very long and slender, resembling the *crassa* parent species in this respect. The ascospores were fairly uniform in size, perhaps somewhat smaller, if anything, than are those of *N. crassa*. Several asci were selected and their spores isolated one by one so that the position of each spore in the ascus was known. The characteristics of the haplonts obtained are given below.

Crassa × (NON-CONIDIAL *sitophila* × *crassa*)$_{20}$

ASCUS A. Ascus A was isolated and left on the surface of the agar still containing its spores. It was found on the following morning that seven of the spores had germinated without heating, a rather unusual occurrence. Tip ends of long germ tubes which could be traced back to individual spores with some degree of certainty were cut off and transferred to tubes. Mycelia 1 and 2 proved to be albinistic and of sex B. Mycelia 3 and 4 produced a profusion of conidia and were proved to be of sex A. It was not possible to be certain as to the identity of the three mycelia which came from spores in the lower end of the ascus. One was albinistic and of sex A. The other two were typical conidial mycelia and were of sex B. Although there exists some uncertainty as to the position of the ascospores giving rise to the particular tips of germ tubes transferred, it is clear that segregations of factors for conidia and for sex had occurred, resulting in ascospores of four types. The spindles of the first and second nuclear divisions in the ascus of *N. crassa* and *N. sitophila* are longitudinal. It may be assumed that the same orientation obtains in the long

slender asci of their hybrids. It is very likely, therefore, that both sets of factors were segregated in the second division, and independently, since albinistic mycelia 1 and 8 were of opposite sex. An example of such a type was not found in the experiments reported previously (**3**). These experiments where albinistic and typical strains of *N. sitophila* were crossed have since been repeated and the results will be given later in this paper.

ASCI B AND B′. The spores from ascus B had been carefully isolated one by one. Cultures were obtained from spores 1, 5, 6, 7 and 8. Mycelium no. 1 was typical in that it quickly developed many pale orange-colored conidia. It proved to be of sex B. Mycelia 5, 6, 7 and 8 were albinistic and were all of the same sex, A. The factors for conidia, as well as those for sex, segregated in the first nuclear division. Although these four albinistic haplonts produced no monilioid conidia, nos. 5 and 6 were of a different type from that of 7 and 8. Their aërial growth was more open and fleecy. The aërial growth of 7 and 8 was rather compact, developing a pinkish ridge at the upper end of the agar slant. If this represents a third character which segregated in the second nuclear division, it could not readily show in case of mycelia like no. 1 because of the profusion of colored conidia. Old cultures of conidial mycelia from ascus D do show decided differences, however. Several transfers were made from cultures 5, 6, 7 and 8, yet the same differences between the pairs 5–6 and 7–8 were maintained. These particular mycelia all being of the same sex, of course, will not mate with each other. By crossing these strains with other albinistic strains it can be determined whether the differences noted are genotypic. Only four spores from ascus B′ germinated. Segregation of both sets of factors occurred in the first nuclear division in the ascus.

ASCUS D. Mycelia 1, 2, 3 and 4 were typical, producing many conidia. They were all of sex A. Cultures of these four clons examined several weeks later showed differences which parallel those mentioned in connection with ascus B. Again both sets of factors separated in the first nuclear division, nos. 5 and 6 being albinistic and sex B.

ASCUS D′. Mycelia were obtained from spores 3, 6, 7 and 8. Mycelium 3 which was of sex B was of the albinistic type of aërial

growth, yet a few conidia were formed in an abnormal way. This type is indicated in table I by the sign ±. It will be discussed briefly in a later paragraph. Mycelia 6, 7 and 8 produced an abundance of monilioid conidia. They were sex A; segregation of both sets of factors in the first nuclear division, but that for conidial factors was incomplete. Ascus L showed the same type of abnormality.

ASCUS L. Mycelia were obtained from spores 1, 2, 3 and 4. They were all albinistic and again we have the type which produces some abnormal conidia, the same as no. 3 in ascus D′. The sex factors segregated in the second nuclear division.

ASCUS M. Mycelia 1, 2 and 7, 8 were non-conidial and sex B. Mycelia 3, 4, 5 and 6 were typical conidial and sex A. Both sets of factors separated in the second division, yet only two kinds of spores were formed. This type of ascus should be compared with ascus A where four different kinds of spores were produced with both sets of factors segregating in the second division.

ASCUS N. Mycelia were obtained from spores 1 to 5. Mycelia 1 and 2 were albinistic; 3, 4 and 5 were conidial; nos. 1, 2, 3 and 5 proved to be sex A, and no. 4 sex B. Evidently some slip occurred in isolating spores 4 and 5. If these two are transposed, it would mean a segregation of the sex factors in the first division and those for conidia in the second.

ASCI O, P AND Q. These three asci belong to the type showing segregation of factors for conidia and factors for sex in the first division. Culture 3 from ascus O is one of the albinistic intermediates.

The data dealing with the various asci just discussed are given in table I.

Even though only a few asci were studied, it is evident that the factors controlling the development of the albinistic non-conidial type of aërial growth, as contrasted with typical conidial mycelium, segregate during the divisions of the fusion nucleus of these interspecific hybrid asci. The fact is also brought out that both sets of factors may segregate together in either the first or second nuclear division in the ascus. Since we are considering only the factors for sex and those for conidia, only two kinds of ascospores were formed in ascus B. The difference between mycelia 5, 6

TABLE I

SEGREGATION OF THE FACTORS FOR SEX AND FOR CONIDIA IN BACK-CROSS ASCI (ALBINISTIC NON-CONIDIAL *sitophila* × CONIDIAL *crassa*) × *crassa*

The first half (S) of each double column shows the sex, A or B, of the particular haplonts which were obtained by germinating spores from that ascus. The second half (C) of each double column shows the production (+ C) or lack of production (− C) of conidia by individual haplonts; albinistic mycelia which produce a few abnormal conidia are indicated by the sign ±. Spores that did not germinate are inclosed ( ).

Ascus No.	A		B		B′		C		D		D′		L		M		N*		O		P		Q	
Spore No.	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
1	B	−C	B	+C	(B	−C)	B	−C	A	+C	(B	−C)	B	±C	B	−C	A	−C	A	−C	(A	+C)	A	−C
2	B	−C	(B	+C)	(B	−C)	B	−C	A	+C	(B	−C)	B	±C	B	−C	A	−C	A	−C	A	+C	(A	−C)
3	A	+C	(B	+C)	B	−C	B	−C	A	+C	B	±C	A	±C	A	+C	A	+C	A	±C	A	+C	(A	−C)
4	A	+C	(B	+C)	(B	−C)	(B	−C)	A	+C	(B	±C)	A	±C	A	+C	B	+C	(A	±C)	A	+C	(A	−C)
5	B	+C	A	−C	(A	+C)	(A	+C)	B	−C	(A	+C)	(B	+C)	A	+C	A	+C	B	+C	B	−C	B	+C
6	B	+C	A	−C	A	+C	(A	+C)	B	−C	A	+C	(B	+C)	A	+C	(B	+C)	B	+C	B	−C	B	+C
7	A	−C	A	−C	A	+C	(A	+C)	(B	−C)	A	+C	(A	+C)	B	−C	(B	−C)	B	+C	(B	−C)	B	+C
8	(A	−C)	A	−C	A	+C	(A	+C)	(B	−C)	A	+C	(A	C)	B	−C	(B	−C)	B	+C	B	−C	B	+C

* Spores nos. 4 and 5 evidently became misplaced.

and 7, 8 is such as to suggest that there were, after all, four genotypically different kinds of spores in this ascus.

It has been proved (**6**) that the sex factors very commonly segregate in the first nuclear division in the ascus of typical *N. crassa*. The factors for sex as well as those for conidia often separate in the second division in the ascus of *N. sitophila* (**3**), but in no case was it found that both sets separated together in the same division. If the segregations are by chance, such types should be met with. The results of further experiments given below show that such is the case.

ALBINISTIC NON-CONIDIAL × TYPICAL CONIDIAL *sitophila*

The Moreaus (**12**) have recently arrayed the evidence to prove that in such ascomycetes as *Sphaerotheca*, *Pyronema* and *Neurospora* the sex organs do not function as such in fertilization, and Gwynne-Vaughan and her associates (**10, 11**) have put forward tentatively the theory of nutritive heterothallism. On the basis of either theory it would be difficult to explain the inheritance of the albinistic character in such a regular fashion, and we ought to find in crosses between albinistic and typical races of *N. sitophila* certain perithecia, or at least certain asci, in which the spores would be either all albinistic or all typical. Partly with this in mind, further experiments with such crosses were carried out and the nature of the spores in several asci from three different perithecia was studied. Strain 56.2, an albinistic strain, sex B, was mated with the typical strain 56.8, sex A (**3,** p. 23). The main purpose being to determine whether each ascus produced some albinistic spores, the exact position of the spores was not important and was not usually recorded. The types of spores obtained from only three of the eleven asci analyzed are noted.

ASCUS A. The eight spores were isolated in order and a culture obtained from each spore. Mycelia 1, 2 and 7, 8 were albinistic and of sex A. Mycelia 3, 4 and 5, 6 were typical and of sex B. Only two different kinds of spores were developed in this ascus. Clearly the factors for conidia and those for sex were segregated in the second division. In each case the +C factor for conidia went with sex B, so that the spores were arranged in the ascus:

two, four, two, with respect to their sex as well as to their conidial factors.

ASCUS L. All eight spores germinated; clons 1, 2 and 5, 6 were proved to be conidial and sex A; 3, 4 and 7, 8 were albinistic non-conidial and sex B. Again both sets of factors segregated in the second division, giving only two types of spores, but in this ascus the spores alternated two and two for both sets of factors.

In ascus H both sets of factors segregated in the first nuclear division. The types obtained from the other asci were either the same as those previously described (**3**) or it was not possible to determine which divisions were the seat of segregations because the positions of the spores were not recorded. If some of these asci developed from the matings between conidial and non-conidial clons are homozygous for conidia, such have not been found. This would indicate that in the production of ascocarps by mating clons of opposite sex, both clons contribute nuclear elements which in some way come together and are represented by the fusion nucleus in the ascus. The results presented above further prove that segregations of factors for conidia and factors for sex segregate independently, either together in the first or the second nuclear division in the ascus, or one set may segregate in the first division and the other set in the second. As yet no evidence has been found of a segregation in the third division.

INTERSPECIFIC HYBRIDS

In crossing *Neurospora sitophila* with *N. tetrasperma* (**8**), it will be remembered that a species regularly having 8-spored asci was crossed with one normally with 4-spored asci. The spores of the former are unisexual, those of the latter are bisexual. The F_1 ascus is long and slender like that of the *sitophila* parent. Eight spores are usually delimited and the ones that mature are about the size of spores of *N. sitophila* or perhaps a little larger. Mycelia from sixty f_1 ascospores were mated in back-cross with each parent species. In no case were asci from such back-cross perithecia homozygous for 4-sporedness as contrasted with 8-sporedness, which were the characters considered. By selecting and again back-crossing with *N. tetrasperma* a segregate no. 209 was obtained which when again back-crossed gave perithecia

with mostly 4-spored asci. The process has since been continued to obtain a unisexual clon 507 which is indistinguishable from unisexual strains of the *tetrasperma* parent, except that for some reason, like 209, it produces a very scanty aërial growth and very few conidia on corn meal agar. If, then, one crosses clon 56.2, the albinistic non-conidial *sitophila*, with 507, he will be making practically interspecific crosses, and in addition have an experiment comparable to Allen's (**1, 2**), especially if he had crossed non-tufted with little-tufted clons.

NON-CONIDIAL *sitophila*, CLON 56.2 × WEAKLY-CONIDIAL "*tetrasperma*," CLON 507

Clon 56.2 was mated in several tube and plate cultures with no. 507. About six weeks later some spores began to mature. Crushed mounts showed that F_1 asci were commonly 8-spored and there was the same large percentage of abortion and slow ripening noted in the first experiments with crosses between typical *sitophila* and typical *tetrasperma* (**8**). Following the usual methods, cultures of seventy-nine mycelia were obtained from f_1 ascospores selected at random. These cultures on corn meal agar were examined thoroughly for production of conidia. Without going into details, it may be said that every mycelium that did not plainly show conidia was repeatedly grown on dextrose agar, potato dextrose agar, white bread, rye bread, corn meal mush and wheat bran mixed, and potato plugs. Such media tend to induce luxuriant aërial growth, but unless a mycelium produces some few conidia on plain corn meal agar, conidia will not be found on these other media.

The following mycelia produced a fluffy aërial growth and quantities of monilioid conidia like typical strains of *N. sitophila:* nos. 4, 8, 9, 14, 35, 36, 39, 40, 43, 48, 56, 59, 61, 68, 69, 73, 75, a total of seventeen, or 21.5 per cent. Certain mycelia produced a scanty growth with few conidia like that of the parent 507. They were as follows: nos. 23, 37, 41, 42, 45, 63, 65, 66, 74 and 80, a total of ten, or 12.6 per cent. The following mycelia were of the albinistic non-conidial type resembling the parent clon 56.2: nos. 1, 2, 5, 6, 10, 12, 13, 15, 19, 20, 21, 22, 26, 27, 29, 31, 32, 33, 34, 46, 47, 52, 55, 58, 60, 64, 67, 71, 76 and 77, a total of thirty,

or about 38 per cent. Those mycelia making scanty aërial growth like 507, but without conidia, were the following: nos. 3, 7, 11, 16, 17, 18, 24, 25, 28, 38, 49, 50, 51, 53, 54, 57, 62, 70, 72, 78, 79 and 81, a total of twenty-two, or 27.8 per cent. In all, fifty-two, or 66 per cent of the seventy-nine mycelia studied, were non-conidial, and twenty-seven, or 34 per cent, were conidial; forty-seven, or 59 per cent, gave good aërial growth, while thirty-two, or 41 per cent, made scanty aërial growth.

It is to be regretted that all eight spores in F_1 asci so seldom mature, making it practically necessary to choose spores at random. Here, just as was the case where albinistic "non-conidial" *sitophila* was crossed with typical conidial *crassa*, only in the reverse, some element has entered to upset the exact 50:50 ratio of conidial to non-conidial mycelia. Only a small percentage of the f_1 spores actually ever mature, and of these not all germinate. The character "scanty growth," coming from the parent 507, which normally ripens quickly, was carried by only 41 per cent of the spores, so that there seems to be no connection between early ripening and scanty growth. This work must be repeated using as one parent a strain of *N. tetrasperma* which produces an abundance of conidia, instead of clon 507.

SEX OF THE F_1 CLONS

Sixty-five of the f_1 mycelia were tested for their sex. The following were sex A: nos. 3, 5, 6, 11, 19, 20, 23, 24, 25, 27, 31, 32, 35, 36, 38, 39, 40, 41, 42, 43, 44, 46, 47, 53, 58, 60, 65, 69, 70, 73, 74, 76, 78, 79, 80, 81, a total of thirty-six. The following were sex B: nos. 1, 2, 4, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 21, 22, 26, 29, 33, 34, 37, 45, 48, 54, 55, 64, 67, 71, 75, a total of twenty-nine. Originally nos. 1 to 48, except nos. 28 and 30, which were lost, and five others were tested. The ratio of the two sexes stood 24 : 27. Several months later thirteen more cultures were tested. They were all sex A except no. 75. The germinating spores, as stated above, were originally selected at random, and the cultures were given their numbers at once, yet beginning with no. 38 we have seven consecutive mycelia all of the same sex A. From no. 70 to 81 the mycelia were mostly sex A; nos. 12 to 18 were sex B. This shows that random selections may sometimes give some peculiar results.

The F_1 ascus is at least diploid and anything like "dominance," which is an attribute manifested by the sporophyte generation, could best be expressed in the form taken by the ascus, which is the only sporophytic structure with definite characters. In addition to being long and slender, the F_1 ascus is 8-spored, and, like the asci of the *sitophila* parent, the ascospores are unisexual, so that f_1 mycelia are "heterothallic." Not one of these mycelia was found to be hermaphroditic. Considering merely the number of spores in the ascus, 8-sporedness is, in a sense, "dominant."

FIRST GENERATION BACK-CROSSES

Fifteen f_1 mycelia, selected because they were albinistic non-conidial, were among those tested for their sex. Twelve were sex B and only three were sex A. Clons 29 and 64, sex B, and clon 47, sex A, were chosen to mate with stock strains of *N. tetrasperma*, S_6 and S_1 respectively, in a series of selections to obtain homothallic albinistic non-conidial clons which would develop perithecia with 4-spored asci like *N. tetrasperma*. The mycelia derived from mating 29 with S_6 will be referred to as set I, those from mating 47 with S_1 comprise set II, and those from $64 \times S_6$ are included in set III.

SET I. The mating $(507 \times 56.2)_{29} \times S_6$ developed ascospores first. Being virtually first generation back-crosses, the asci contained variable numbers of spores, mostly from two to six, varying from 20 to 60 μ or more in length. All spores cut out seemed to mature. The larger spores must have contained several nuclei, probably in this case differing genotypically. Sowings were made and a number of germinating small spores were selected because, being very small, they must be unisexual. The inheritance represented in a giant spore can be determined by plating out the conidia from its mycelium. The purpose of these experiments, however, was principally to obtain albinistic non-conidial haplonts. Their mycelia differed greatly as to color, types of aërial growth, and production of conidia. These differences were heightened for some and decreased for others as the cultures aged (PLATE 4). The notes given below were made when the cultures were about ten days old, with modifications later as necessary.

1. Non-conidial; albinistic, whitish fluffy aërial growth.
2. Conidial; at first whitish to light brownish aërial growth, then turning a beautiful bright saffron pink. Later transfers did not show such striking colorations.
3. Non-conidial; brownish fluffy growth, later fading to whitish.
4. Non-conidial; pale light brownish fluffy growth.
5. Non-conidial, and the same type as no. 4.
6. Conidial; light brownish aërial hyphae, then light orange-colored like *N. sitophila*.
7. Non-conidial; whitish, fleecy and albinistic growth.
8. Non-conidial; somewhat albinistic, then slightly orange to brownish growth.
9. Non-conidial; pinkish felty surface growth.
10. Non-conidial; scanty aërial growth.
11. Conidial; rather scanty, felty aërial growth.
12. Non-conidial; at first pale fluffy aërial growth, then orange-brownish.
13. Non-conidial; scanty growth.

Eight of the non-conidial mycelia were tested out for their sex; nos. 1 and 13 were sex A and 3, 4, 5, 7, 8 and 9 were sex B.

SET II. $(507 \times 56.2)_{47} \times S_1$. Mycelium 47, being sex A, was mated with stock clon S_1, sex B of *N. tetrasperma*. Twenty-one mycelia were obtained from the unisexual ascospores. The characters of the first twelve are given below (see also PLATE 4). The other nine, for some reason, did not show much aërial growth and were not studied fully.

1. Conidial; light orange-colored aërial growth, turning to reddish orange later; conidia much more abundant than most strains of *N. tetrasperma*.
2. Non-conidial; pale pinkish aërial growth, tending to albinistic.
3. Conidial; not very heavy orange-colored growth.
4. Conidial; pinkish-orange, conidia persistent, later turning darker orange-colored to brownish.
5. Non-conidial; a typical albinistic growth.
6. Non-conidial; very scanty aërial growth.
7. Non-conidial; at first a scanty aërial growth, later becoming fluffy to felty.
8. Conidial; scanty aërial growth, becoming felty.
9. Conidial; rather pale orange-colored, like Arl. 10 strain of *N. sitophila*. Good aërial growth.
10. Conidial; pale fluffy growth with many conidia later.
11. Non-conidial; scanty aërial growth.
12. Non-conidial; very little aërial growth.

Again one notes that when a mycelium produces conidia, it does so with vastly greater abundance than does either the original conidial parent, 507, or the immediate back-cross parent S_1.

Clons 7, 12 and 20 were proved to be of sex A and 1, 2 and 5 were of sex B. The others were not tested for sex.

SET III. $(507 \times 56.2)_{64} \times S_6$. Twelve mycelia were obtained as usual by germinating the small unisexual ascospores from this mating. Their characters are given here to further prove that, so far as types of aërial growth, production of conidia and coloration are concerned, segregations were not such as to produce only the two kinds represented by their immediate parentage. No doubt, however, if one analyzed the total inheritance represented by the eight nuclei from any particular ascus he would find for any one character four nuclei of one kind and four of the contrasted character.

1. Non-conidial; very little fluffy growth, but a rather compact, pinkish ridge of growth at the top of the agar slant.
2. Non-conidial; same as no. 1.
3. Non-conidial; growth like no. 1.
4. Conidial; bright orange-colored growth, conidia like *N. sitophila*.
5. Conidial; great abundance of orange-colored conidia.
6. Non-conidial; very scanty aërial growth.
7. Conidial; rather pale faded orange-colored growth.
8. Non-conidial; very scanty growth.
9. Non-conidial; much like no. 8.
10. Non-conidial; somewhat albinistic but rather limited aërial growth.
11. Non-conidial; little aërial growth.
12. Non-conidial; like no. 11.

Tested for their sex, nos. 1, 8, 9, 10, 11 and 12 were of sex A type, and 2, 3, 4 and 6 were sex B. The other two were not tested.

Thirty-seven haplonts included in sets I, II and III were examined very carefully for production of conidia. Twelve, only, produced conidia. Without counting the nine apparently non-conidial mycelia discarded from set II, only 32 per cent were conidial. Twenty-four mycelia were tested for their sex. Eleven were sex A and thirteen were sex B. As noted previously, several of these mycelia produced conidia in amounts greatly in excess of what either no. 507 or S_6 ever produces on the same medium. Nowhere in any strains of either species has such a bright saffron pink and orange color as shown, for example by clon 2 in set I, been observed. This would be something entirely new if it continued to keep these colors in future transfers. For some reason, the few transfers made do not show such brilliant colora-

tions. Clon 1 in set II retains its deep colors in subsequent transfers.

PROGENY OF MATING $\{(507 \times 56.2)_{29} \times S_6\}_8 \times S_6$

Comparing crushed mounts of perithecia from cultures comprising sets I, II and III, it was found that the culture in which S_6 was mated with clon 8 of set I gave asci fairly regularly 4-spored, but occasionally an ascus would have five or six spores. Several asci containing mature spores were selected and their spores isolated one by one. All four spores in several asci germinated. The characters of the mycelia derived from some of these asci will be described first, beginning with the asci which appeared to be fairly regular as to segregations of factors for sex and for conidia. Later a table will be given to include all asci studied.

The peculiar orientation of the spindles in the three nuclear divisions in the 4-spored ascus of *N. tetrasperma* has already been described in some detail (**7**). A spore will ordinarily contain one nucleus of each sex when cut out, making it bisexual and its mycelium totipotent. It is only rarely, or about 1 per cent of the time, that both nuclei in a binucleate spore are of the same sex. Such a distribution of the nuclei is easily explained. If the sex factors segregate in the second nuclear division and, while the four reorganized nuclei are separating on their spindles, the two bearing factors for sex B move one way and the other two bearing sex A factors move in the opposite direction, then two nuclei, each containing sex A factors, are included in each of two spores. The other two spores would each be provided with two sex B nuclei. Irregularities in spindle orientation such as must be expected in hybrids could also enter and affect the position and thus the distribution of the nuclei which are included in particular spores.

Where possible in this work two sets of cultures were obtained from germinating spores; one by transferring tip ends of germ tubes and the other by transferring the ascospores with some of the remaining growth. As a spore begins to germinate, the nuclei divide rapidly so that the germ vesicles and their branches contain several nuclei each. Where the two original nuclei differ genotypically, one is never certain that in transferring a single

germ tube tip he gets all of the inheritance represented by the spore. This will be seen by referring to figures of nuclei in ascospores and conidia (**9**).

ASCUS G. The four spores, isolated in order, germinated. Mycelia nos. 3 and 4 appeared not to produce conidia and the cultures showed young perithecia on the third day. Nos. 1 and 2 were conidial and began to show perithecia on the fifth day. Later tests indicating that 3 and 4 produced few if any conidia, it became necessary to prove that both nuclei in spore no. 1 carried +C factors for conidia, and to check this up by proving the same thing for spore no. 2. Following the method previously described (**9**), twenty-nine mono-conidium cultures were obtained from mycelium 1G. Of these mycelia nineteen were bisexual and produced an abundance of perithecia. Ten mycelia, or 35 per cent, were unisexual, four of these being sex A and six sex B. When one plates out single conidia from normal *N. tetrasperma* he frequently gets as high as 35 per cent unisexual mycelia. Mycelia 6 and 29 produced quantities of orange-colored conidia; nos. 9, 12, 19 and 20 produced a scanty aërial growth and only small quantities of conidia. These haplonts all happened to be sex B. The other four were sex A and produced only a very few conidia, scarcely visible even with a hand lens. Just how many of these differences are merely phaenotypic or are connected with the sex of the mycelia is a question. The bisexual mycelia showed comparable differences in the amounts of conidia produced.

Twenty-five mono-conidium cultures were obtained by plating out conidia from mycelium no. 2G. Here again there were three kinds of mycelia as regards their sex and all likewise produced conidia. Fifteen mycelia were bisexual and ten were unisexual. Of the latter, four were of sex A and six were sex B. Clons 20 and 25, both sex B, developed fairly large quantities of orange-colored conidia. The other eight produced only a comparatively few. Since none of the twenty unisexual haplonts derived by plating out conidia borne on mycelia 1G and 2G failed to produce conidia and as the sexes A and B were both represented it is evident that there was a segregation of the factors for conidia in ascus G. The eight nuclei were so distributed that the four

carrying the +C factors for conidia were all included in spores 1G and 2G. With segregation of the sex factors in the first nuclear division and those for conidia in the second division one can readily see how such a distribution can come about if the spindles are oriented the same as they are in normal *N. tetrasperma*. During the second nuclear division the spindles lie as though in conjugate division and oblique to the long axis of the ascus. In segregating, the +C factors for conidia pass to the nucleus at the upper end of the spindle in each case. A third division in which the spindles are more or less transverse then occurs. This leaves the four nuclei carrying +C factors in the upper end of the ascus and they will all be included in spores nos. 1 and 2.

ASCUS O. The four spores germinating, two sets of cultures were readily obtained. Mycelia 1, 2 and 3 produced conidia, and perithecia quickly developed; no. 4 in both sets appeared to be non-conidial. The one derived from a "tip" transfer produced perithecia, but the culture from the ascospore remained sterile for a long time. Very likely a few conidia which were overlooked were produced by no. 4. A discussion of weakly bisexual mycelia will be taken up later on. Segregation of the factors for conidia and those for sex could have occurred in either the first or second nuclear division.

ASCUS Q. Each of the four mycelia produced conidia, and in due time matured perithecia, showing that each spore contained nuclei of both sexes and one nucleus of each carried the +C factor for conidia. Tests were not made to learn whether all four nuclei of either sex carried such factors.

ASCUS R. All four spores germinated and again two sets of cultures were obtained. The four mycelia in each set produced conidia. Nos. 1 and 2 were weakly bisexual, developing comparatively few ascocarps. Nos. 3 and 4 were strongly bisexual. A great number of ascocarps matured within ten days. Evidently the segregations for conidia were regular, a point easily proved by plating out the conidia from the four cultures, or by germinating the small unisexual ascospores. There was nothing in the appearance of any of the four cultures to indicate the hybrid nature of the ascocarps.

Ascus D. Only spores nos. 1 and 2 germinated. Both mycelia produced conidia and perithecia appeared in both cultures, discharging ascospores freely on the eighth day.

It is clear from the foregoing account that each of the spores from the five asci described contained nuclei of both sexes, and that, with the exception of spores 3 and 4 from ascus G, the +C factor for conidia was carried by at least one nucleus in each spore. No attempt was made to induce conidial formation by mycelium 4 from ascus O. It may have been weakly conidial. Spores 1 and 2 in ascus G were the only ones in which both nuclei carried conidial factors. There was a suggestion that their mycelia produced more conidia than was the average for mycelia where only one of the two nuclei in the spore carried the +C factor. This would need much more confirmation, as there is apt to be much irregularity in the quantity of conidia produced by any mycelium under slightly different conditions.

Blakeslee, Burgeff and others, working with the Mucoraceae, have noted that occasionally a monospore mycelium derived from a primary sporangium remains neutral, not reacting with either tester strain. Various explanations have been put forward to account for such "neutrals." Comparing the reactions of various mycelia, these authors have found all grades of fertility or strength of sexual reaction. Just such irregularities have been noted from time to time in testing clons of *Neurospora* species. Some of the results of crossings being described here would suggest that in addition to, or instead of, weak sexuality, we would have to consider the possibilities of self-sterilities and incompatibilities. For example, the mycelia derived from spores from asci J and L behaved so erratically in tests for their sexuality as to indicate at first very poor technique in making transfers, or contamination of cultures by mites or otherwise. Against such an assumption may be cited the fact that no perithecia have ever appeared in stock tester strains of each of three species where transfers are constantly being made and old cultures are often kept around the laboratory for several months. Some details of the culturing done in testing out mycelia derived from asci J and L are given below, even though it was thought best at one time to discard the whole series as merely illustrating poor tech-

nique. Results of later experiments, as will be seen, account fully for all the apparent irregularities here reported, and reveal some interesting characteristics of these interspecific hybrids.

ASCUS J. All four spores germinated. Bits of agar each bearing a spore were transferred to another plate and placed in order at about equal distances around the periphery of the petri dish. After the germ tubes had grown out and branched, tip ends from each transplant were cut off and transferred for cultures in set I. The ascospores themselves, together with short pieces of attached growth, were transferred to tubes for set II. In each case there was left in the plate enough growth to represent each spore. Mycelia grew out meeting along four lines, forming four sectors. After about a week perithecia began to appear all over sector no. 4, and then later a few formed at the outer end of the line where mycelia 2 and 3 met, followed by a few scattered irregularly here and there over sector 3, especially adjacent to sector 4. Even after six weeks none appeared on sectors 1 and 2. In the meantime, two other plates were inoculated, one with the inocula in the same relative positions as before, the other with nos. 2 and 3 transposed. Not a single ascocarp developed in the first culture. A few did finally form in sector 3 in the other culture. Mycelia 2 and 4 were planted in another plate culture. Some few perithecia finally formed at one point where the no. 4 transplant was placed.

Turning to the tube cultures, we find that all four mycelia in both sets I and II developed some conidia. The original cultures in set II kept for seven months never developed any ascocarps, and so long did all the cultures in both sets remain free of perithecia that it was thought here was a clear cut case of segregation for sex in such a way as to throw two nuclei of the same sex in each spore. This would mean that by mating mycelia nos. 1 to 4 in all possible combinations, one could readily determine which spores had contained two nuclei of sex B and which two nuclei of sex A. The first plate culture had strongly suggested that mycelium 4 must be sex A, because in *N. tetrasperma* matings of S_1 and S_6, perithecia always form on S_6, which is sex A, and not on S_1, which is sex B (PLATE 7, FIG. 17).

Ordinarily in making tests for sex, perithecia will make their

appearance within from three to six days. If the strain in question does not react with either tester strain within this time, another test is made, but the former cultures are kept under observance until the question is settled one way or the other.

The following combination cultures were made at various times. If, even after several months, perithecia ever appeared in any culture, this is indicated by the sign "+." The sign "−" indicates that no perithecia developed. Tester strains S_6 and S_1 are, as noted above, the stock unisexual tester strains of *N. tetrasperma*.

TEST I

$1J \times S_1 = -$;	$1J \times 2J = -$;	$1J \times S_6 = +$
$2J \times S_1 = -$;	$1J \times 3J = +$;	$2J \times S_6 = +$
$3J \times S_1 = +$;	$1J \times 4J = +$;	$3J \times S_6 = (-) +$
$4J \times S_1 = +$;		$4J \times S_6 = (-) +$

Since cultures $3J \times S_6$ and $4J \times S_6$ did not show perithecia at first, the results were thought to prove conclusively that 1J and 2J were of sex B and 3J and 4J were of sex A. Another test was made when, later, perithecia did show up in the tubes where 3J and 4J were grown with S_6.

TEST II

$1J \times S_1 = -$;	$1J \times S_6 = +$
$2J \times S_1 = -$;	$2J \times S_6 = +$
$3J \times S_1 = +$;	$3J \times S_6 = -$
$4J \times S_1 = -$;	$4J \times S_6 = +$

Since the second test shows 1, 2 and 4 as sex B and no. 3 only as sex A, a third test was made as follows:

TEST III

$1J \times S_1 = -$;	$1J \times S_6 = +$;	$1J \times 2J = -$;	$2J \times 4J = -$
$2J \times S_1 = -$;	$2J \times S_6 = +$;	$1J \times 3J = -$;	$3J \times 4J = -$
$3J \times S_1 = +$;	$3J \times S_6 = +$;	$1J \times 4J = -$;	
$4J \times S_1 = -$;	$4J \times S_6 = +$;	$2J \times 3J = -$;	

With the exception of $3J \times S_1$, all combinations in this test showed that the four mycelia were now of sex B. By this time it was noted that cultures 2 and 4 of the originals in set I showed a few perithecia, and one of the sub-cultures from 3J also bore perithecia. This was about three months after the first cultures were obtained. The four cultures in set II derived from asco-

spores were still devoid of any signs of perithecia. Transfers were made from this set and new tests made as follows:

TEST IV

$1J \times S_1 = -$;	$1J \times 3J = -$
$2J \times S_1 = -$;	$1J \times 4J = -$
$3J \times S_1 = -$;	$2J \times 3J = +$ (one perithecium)
$4J \times S_1 = -$;	$2J \times 4J = +$ (one perithecium)

The results were again irregular.

While testing mycelia from ascus J, the progeny from ascus L were also under investigation.

ASCUS L. Three sets of cultures, I, II and III, were obtained from the four spores from ascus L. A few conidia were developed in each culture, but only a very scanty aërial growth was made on corn-meal agar. The first tests for the sexuality of the mycelia were made in petri dish cultures where the four were grown together. Placed at equal distances around the periphery, the four mycelia met along four lines, forming four sectors, as is usual, but at the end of ten days no fruit bodies had formed in any one of the four different plates. This indicated that all four mycelia were of the same sex. These cultures were discarded and further tests made in tube cultures, as follows:

$1L \times S_1 = -(+)$;	$1L \times S_6 = +$
$2L \times S_1 = -$;	$2L \times S_6 = +$
$3L \times S_1 = -$;	$3L \times S_6 = +$
$4L \times S_1 = -$;	$4L \times S_6 = +$

Since perithecia formed quickly in all combinations with S_6, it was clear that the four mycelia were of the same sex, A, confirming the petri dish tests. The combination $1L \times S_1$ did not show perithecia until about six weeks later. Hoping to prove that here was a case where all of the eight nuclei in an ascus were of the same sex, the mycelia were grown together in all possible combinations. The results were again negative, further proving that the four mycelia were sex A. The original cultures in sets I, II and III, not showing any perithecia after being under observation for about two weeks, had been set aside and sub-cultures used for testing. Now, after several more weeks it was found that nos. 1 and 4 in each set showed a few mature ascocarps. Choosing 1 and 4 from among the sub-cultures showing no fruit bodies,

other tests were made, using various tester strains of *N. sitophila* and unisexual hybrid strains. All of these gave only proof that nos. 1L to 4L from the sub-cultures were of sex B.

With the idea that if each spore contained nuclei of both sexes, but that perhaps the sex A nucleus was acting in some incompatible capacity, attempts were made to separate the two by plating out the conidia. About twenty single conidium mycelia were obtained from 1L. None of these produced perithecia in culture. They were all proved to be sex B. Fourteen single conidium strains of 2L were tested against S_1. No fruit bodies were formed. Several tested against S_6 gave perithecia. While

TABLE II

Ascus	Spore	Conidia	Sex	Remarks
A	1	+C	A + B	Scanty growth, weak sexuality.
	3	+C	A	Scanty aërial growth.
	4	−C	A + B	Very strong sexuality; ripe ascospores within eight days.
B	3	+C	B	Only one spore germinated.
C	1	+C	B	Some few large sterile bodies.
	3	+C	B	
	4	+C	A + B	Weak sexuality.
D	1	+C	A + B	Strong sexuality; matured ascospores within eight days.
	2	+C	A + B	Strong sexuality; matured ascospores within eight days.
E	2	+C	A	Culture produced one large sterile body.
F	1	−C	A(+B)	Strongly sex A; very weakly A + B.
	2	−C	A + B	Very weak sexuality.
	3	+C	A + B	Fairly strong sexuality.
	4	+C	B	
G	1	−C	A + B	Very fertile.
	2	−C	A + B	Very fertile.
	3	+C	A + B	Slow development but fairly fertile.
	4	+C	A + B	Slow development but fairly fertile.
H	1	+C	B	May have had two sex B nuclei.
	2	+C	B	Small spore.
	3	−C	A	Probably had two sex A nuclei.
	4	+C	A + B	Matured many ascocarps.
	5			Fifth spore small; did not germinate.
J	1	+C	B	
	2	+C	B(+A)	See text for explanation.
	3	+C	A(+B)	See text for explanation.
	4	+C	B(+A)	See text for explanation.

TABLE II (Continued)

Ascus	Spore	Conidia	Sex	Remarks
K	1	+C	A + B	Very weakly bisexual; a few perithecia after several weeks.
	2	+C	A + B	
L	1	+C	B(+A)	Fairly strong sex B; slowly and weakly bisexual.
	2	+C	B	
	3	+C	B	
	4	+C	B(+A)	Fairly strong sex B; very slowly and weakly bisexual.
M	1	+C	A(+B)	Very slowly and weakly bisexual; strongly sex A.
	4	−C	B	
N	1	+C	A	
	2	+C	A(+B)	Weakly bisexual; strongly sex A.
	3	+C	A	
O	1	+C	A + B	
	2	+C	A + B	
	3	+C	A + B	Weakly bisexual.
	4	−C	A + B	Conidia very few if any.
P	1	+C	B(+A)	Approaches neutral, but was bisexual.
	2	+C	B(+A)	Approaches neutral, but was bisexual.
Q	1	+C	A + B	
	2	+C	A + B	
	3	+C	A + B	
	4	+C	A + B	
R	1	+C	A + B	Only a few perithecia.
	2	+C	A + B	Not very strong sexually.
	3	+C	A + B	Many perithecia.
	4	+C	A + B	Great abundance of perithecia.

mycelia 1L and 4L in the three sets of original cultures produced perithecia all other tests seemed to indicate that the mycelia were unisexual and reacted rather normally but not strongly as sex B.

The nature of the mycelia from nine other asci where not all of the spores germinated was also studied, but without extensive tests for their sexuality. Several mycelia gave promise at first of being neutrals, but all finally produced some perithecia either alone or in a mating with tester strains of one or the other sex. The results are given in the above table. Under the column headed "Sex," A + B means that the mycelium produced perithecia in culture. A(+B) or B(+A) means that ordinary tests

would show the mycelium to be unisexual, but when allowed to grow alone a long time in culture it would finally develop at least one or two good perithecia. Such mycelia will, for convenience, be referred to as "weakly bisexual."

It would be strange if there were not some nuclear abortion before spore delimitation in these hybrids. An ascus might contain four spores apparently of equal size, yet one of them have only one of the two nuclei which it would have had if the other nucleus had not aborted. Judging from what we know of nuclear behavior in the parent species *N. tetrasperma*, a uninucleate spore will be very small, because there is the companion uninucleate spore to require spore plasm for its growth also. With the one nucleus aborted the survivor of the pair is free to appropriate enough extra spore plasm to increase its size to that of the other spores with two nuclei. Such a condition could be readily proved if every nucleus carried +C factors for conidia. Should, however, one of the two nuclei in a spore be weakly, say, sex A, or in some way have taken on an incompatibility in relation to its natural mate nucleus, sex B, no perithecia would develop in a culture from the mycelium from this spore. Grown with some other unisexual sex A mycelium, perithecia would be formed. Grown with some other unisexual sex B mycelium, no fruit bodies would form because of antagonism between the normal sex B nuclei contained in each of the mycelia in the culture. The table does not show fully how irregular, inconsistent and inconclusive the results of the tests for sexuality really were. For this reason the work was repeated starting with fresh cultures of the mating $\{S_6 \times (507 \times 56.2)_{29}\}_8 \times S_6$. The progeny from each ascus studied will be briefly described. With the exception of ascus M′, which had five spores, each ascus selected had only four spores, all about the same size, suggesting that each contained at its origin two nuclei of opposite sex.

ASCI A′, D′ AND E′. The spores from each of these three asci all germinated, and their mycelia produced conidia and perithecia with no suggestion of irregularities. When incubated at 27° C. fruit bodies began to form about the fourth day.

ASCUS B′. Cultures were obtained from spores 1, 2 and 3. Mycelia 1 and 2 produced conidia; no. 3 was apparently non-

conidial. No cultures on other media were made to test this out further. Perithecia did not begin to appear in cultures 1 and 3 until the seventh day; no. 2, failing to produce perithecia at this time, was thought to be unisexual. Tested against S_1 it reacted strongly as sex A. This was further confirmed by the fact that no ascocarps formed in the culture where it was grown with S_6. At the end of a month perithecia began to form in the original culture. Later cultures on corn-meal agar to which had been added a little potato dextrose agar produced numbers of perithecia but only after considerable delay. This illustrates again how necessary it is in this work to keep the culture under observation for a long time; otherwise one may be misled into thinking the mycelium unisexual, not only because no ascocarps have been formed but also because of its strong reaction with the one or the other tester strains. Mycelium 2B′ was in reality bisexual, being provided with nuclei of both sexes at the origin of the ascospore. Some element of infertility or incompatibility is present to prevent the full expression of the sex B nuclei until after a long time has elapsed.

Ascus C′. The four mycelia derived from ascus C′ all matured a few conidia. Nos. 3 and 4 developed perithecia on the fifth day. Culture 1 first showed ascocarps on the eighth day; otherwise it is strongly bisexual. Culture 2 and several subcultures from it have remained sterile for four months. There seems no likelihood of any perithecia forming at this late date. Tested several times against S_1 it always reacts strongly as sex A. Grown with S_6 no fruit bodies ever form. By every test made mycelium no. 2 was unisexual. The other three mycelia derived from the same ascus were bisexual. Degeneration of one of the eight nuclei before spore formation would account for this irregularity.

Ascus F′. The four mycelia bore conidia; nos. 3 and 4 were strongly bisexual; nos. 1 and 2 produced only a few perithecia and these very slowly. Their reactions when the four mycelia were all grown together in a petri dish culture are suggestive. The sectors occupied by 3 and 4, being adjacent, covered half of the plate. This part was soon thickly dotted all over with ascocarps, while none had appeared on sectors 1 and 2 by the

eighth day. Tested in tube cultures against S_1, no. 2 reacted strongly as sex A. Cultured alone in tubes some few perithecia always develop in due time, however, showing the dual nature.

ASCUS G′. Each of the four spores germinated. Mycelia 1, 2 and 4 produced many more conidia than is usual for these hybrids; no. 3 developed about the average quantity. A few perithecia were finally matured in cultures 1 and 2. No ascocarps were formed in cultures 3 and 4 during four months. The few tests made indicate that both 3 and 4 are of sex B; in which case each ascospore could have received only one sex B nucleus. It may be that we have here merely another example of self sterility or strong incompatibility. This would be difficult to prove unless the +C factors for conidia were distributed among the eight original nuclei in the ascus so that they would be carried by two nuclei of each sex. The production of microconidia by the albinistic strains of *N. sitophila* should not be forgotten (**3**). Should such asexual reproductive bodies be developed by the hybrid mycelia with which we are now dealing they could be isolated. Theoretically, in the case under discussion the microconidia could well be of sex A instead of sex B. Many of these mycelia appear at first to be unisexual in culture and then after several weeks or months slowly develop perithecia. This warrants caution in coming to hasty conclusions as regards their sex nature.

ASCUS H′. One spore failed to germinate. The mycelium from no. 1 appears to be unisexual, sex B; nos. 2 and 3 proved to be bisexual. All three produced conidia. If further tests on other media likewise prove no. 1 to be unisexual, spore 4 should have been unisexual and sex A; whether its mycelium would have produced conidia involves too much speculation.

ASCUS K′. Only spores nos. 1 and 2 germinated. Both mycelia were conidial. After about ten days a few ascocarps began to appear in culture 1. Culture 2 continued sterile for several days. Tested against S_1, both react strongly as sex A. Tested against S_6 the cultures are either sterile or produce fewer perithecia than either one does by itself. Culture 1 is at first only weakly bisexual but finally after about two months many good perithecia develop.

ASCUS M′. This is the first ascus with five spores from which the five mycelia have been analyzed. Some of the details will be given because they show clearly segregations for conidia and definite distribution of the nuclei carrying conidial factors and throw further light on the question of "weakly bisexual" mycelia. Spore 1 was slightly larger than normal. Spores 2 and 3 were very small spores, about 22 μ long. Spores 4 and 5 were of average size for bisexual spores. All the spores except no. 3 germinated within a few hours after they had been heated. It was not until a day later that no. 3 began to germinate. It was carefully transferred to a plate culture where the mycelium grew very slowly for two days; then it began to grow normally. This fact will be referred to again. Two sets of cultures were obtained, tip ends of germ tubes being transferred for set I and the remaining growth including the ascospores themselves for members of set II. Mycelia 1, 2, 4 and 5 developed conidia; no. 3 was grown on several kinds of media but in no case could any conidia be found. Cultures 1, 4, and 5 in both sets as well as in subcultures produced perithecia rather slowly but in some quantity. They would not be classed as strongly bisexual. Cultures 2 and 3 remained sterile. This was to be expected of the mycelia from the two small spores in a 5-spored ascus. In studying the cytology of asci of *N. tetrasperma* (7) nothing was found to indicate just why occasionally only one nucleus is included in each of two spores. One would naturally suspect some incompatible repulsion or weak sex attraction as responsible for the irregularity. No test has heretofore been made to ascertain whether the two small spores in such asci are of the same sex or not. It is of interest therefore to learn from the results of tests with our two mycelia that no. 2 is sex A, producing many perithecia in cultures with S_1. Grown with S_6 no fruit bodies are formed. Mycelium 3 was proved in the same way to be sex B. Should there be any incompatibility relationship it did not show very strongly or conclusively when clon 2 was grown in culture with no. 3. Such combination cultures developed about the same number of ascocarps as did the bisexual mycelia derived from this ascus, but not nearly so many as when each was mated with the tester strain of the opposite sex. Further work would be necessary,

however, to confirm this statement, as it is an important point which should be settled on the basis of a larger number of cultures.

Four of the five mycelia obtained from ascus M′ developed conidia. We have here material adaptable for a study of several interesting questions. The writer (**3**) has discussed the origin of the albinistic non-conidial race of *N. sitophila* with which these experiments were begun. This new race was due to a "saltation," or some sort of somatic segregation or non-distribution occurring in the Arl. 10 strain. It was proved that certain conidia develop into mycelia which are incapable in turn of producing conidia, at least to any extent. Normal bisexual mycelia of *N. tetrasperma* produce three kinds of conidia, *i.e.*, bisexual, unisexual sex A, and unisexual sex B (**9**). It may be asked, what is the nature of the conidia produced by our mycelia 1, 4 and 5 derived from the bisexual hybrid ascospores in ascus M′, each spore having one nucleus which does not carry the +C factors determining conidium production? Shall we have some bisexual conidia? Will some of the conidia be unisexual and if so will some of them be of one sex and some the other, as in case of normal *N. tetrasperma?* Or will some of these conidia develop into non-conidial mycelia because they were provided with only the nuclei lacking the +C factors for conidium production? Since four of the five spores in ascus M′ developed conidial mycelia and as this ascus is a hybrid from a cross between non-conidial and conidial races, we assume segregation of factors for conidia. Relative to their sex, which four nuclei carry the +C factors for conidia and how can this be proved? These are a few of the questions that occur to one from time to time as he studies this interesting material. The answers to some of them appear in the results of the experiments recorded below.

The nature of single conidia derived from mycelium 1M′. Without taking special precautions, twenty-one single conidium cultures were obtained by the usual method. Coming from a bisexual parent mycelium it is rather strange that only one culture produced any perithecia. Culture 20 did form a few fruit bodies, but all of the other twenty have remained without ascocarps, up to date. These cultures are now about four months old. Tested thoroughly against tester strains S_1 and S_6 it was proved that all

were of sex A class. Clons 5, 11, 13 and 17 were rather weak in their reaction, but the others were very fertile with S_1 under the same conditions. Not one of the twenty-one mycelia failed to produce conidia, although certain ones developed only comparatively few.

Single conidium mycelia from 4M′. Twenty-five cultures were obtained from single conidia plated out from culture 4M′. Mycelia 4, 5, 13, 16 and 19 were dwarfs which grew so slowly that they could not be tested out for their sex. The original cultures from the other twenty conidia never produced any ascocarps. Tested out for their sex it was proved without a doubt that they were all unisexual and again all of sex A class. Furthermore, each mycelium produced conidia.

Single conidium mycelia from 5M′. Twenty-five cultures were obtained from single conidia from 5M′ which, like nos. 1 and 4, is bisexual. Again several of these mycelia grew very slowly, but by transferring them to dextrose agar they became invigorated so that they could be studied. Every one of the twenty-five mycelia produced conidia, but no perithecia. Tested for sex, it was again proved conclusively that they were all of sex A class.

It is not at all surprising to find that all four sex A nuclei in ascus M′ carried the +C factors for conidia and all sex B nuclei were non-conidial. On the other hand, it is very remarkable that of the cultures derived from single conidia developed on the bisexual mycelia, 1M′, 4M′ and 5M′, only one out of sixty-six produced ascocarps. It was desirable in these experiments not to be too particular in selecting the conidia, being careful only to avoid transferring hyphal fragments. The results are all the more conclusive on this account as showing that only those nuclei carrying the +C factors for conidia are included in a conidium. Any conidium having non-conidial as well as conidial nuclei would be bisexual and its mycelium would produce perithecia. Only one such culture was obtained among the sixty-six studied. This one was due no doubt to the selection, as noted previously, of a proliferating hyphal fragment rather than a germinating conidium.

Three kinds of conidia are formed on the normal bisexual or hermaphroditic mycelia of *N. tetrasperma* because of the unequal

distribution of the sex A and sex B nuclei. The Arl. 10 unstable sex B race of *N. sitophila* produces two kinds of conidia, "typical" and "albinistic." The bisexual mycelia 1M′, 4M′ and 5M′ go back for their real origin to these same parents, *N. tetrasperma* and the saltant non-conidial races of *N. sitophila;* yet we find quite a different rule followed in conidium formation. At the end of the third division in ascus M′ the four nuclei containing the +C factors are all of sex A class, and only one of these is included in an ascospore. Spores 1 and 2 from ascus G, although developed in a hybrid ascus with the same parentage as ascus M′, correspond to normal *N. tetrasperma* ascospores in that they contain one nucleus of each sex and each nucleus carries the +C factors. In forming conidia their mycelia behave like *N. tetrasperma* in developing three kinds of conidia. The other four nuclei in ascus G were non-conidial.

These results help to explain the peculiar behavior of mycelia obtained from other asci, notably asci J and L, where all of the mono-conidial mycelia obtained by plating conidia were unisexual and sex B. The delayed and erratic production of ascocarps in the original cultures derived from their ascospores was no doubt due to certain incompatibilities or types of self-sterility. The continued sterility of most of the sub-cultures, however, might be due to transferring only conidia, which would mean inclusion of only sex B elements. Such an explanation is a very doubtful one, as sub-cultures were usually made by transferring bits of agar containing mycelium rather than merely conidia. It seems more likely that in the growth processes the nuclei of one sex divide more rapidly than do those of the other sex, so that in certain areas of growth the hyphae contain only nuclei of one sex. This might be only a temporary condition, but if transfers were made from such a place a unisexual strain would be started. Whatever may be the true explanation of the irregularities in sexual reproduction and conidium formation encountered in the culture experiments described in this report, it is clear that the rôle of the cytoplasm and nuclei respectively in these processes is a subject for a more extended study.

HERMAPHRODITIC NON-CONIDIAL 4-SPORED HYBRIDS

One of the aims in making the interspecific crosses was to obtain a bisexual or hermaphroditic strain with a fluffy albinistic non-conidial aërial growth like strain 56.2, which was the 8-spored *sitophila* parent. It was also desired that the hybrid should have 4-spored asci like *N. tetrasperma*. It has been shown that strains no. 4, ascus C, and nos. 3 and 4, ascus G, were bisexual, non-conidial, and the asci in their perithecia were fairly regularly 4-spored. Their aërial mycelia, however, were rather scanty. It was not at all difficult to keep the two characters, albinistic and non-conidial, together in the back-cross hybrids between no. 56.2, *sitophila*, and conidial *crassa*. When, however, *crassa* with its fluffy aërial growth and abundance of conidia was replaced with strain 507 with its scanty aërial growth and few conidia, difficulties arose. The chances were very much against getting together in the same ascospore two nuclei of opposite sex, each lacking +C factors for conidia and each carrying factors for the luxuriant aërial mycelium. Strain S_6 was used as the 4-spored back-cross parent instead of no. 507. It likewise produces very little aërial growth and not very many conidia. S_6, being straight *tetrasperma*, may be presumed on this account to be more regularly 4-spored than the segregate no. 507. In the endeavor to learn something of the nature of the "weakly" bisexual strains and the way segregations of the factors for sex and for conidia were occurring in the 4-spored hybrid asci, the fluffy albinistic feature was not made the main basis for the selections. A segregate with the desired combination of characters listed above could have been obtained more readily in another way. From the cultures which gave the most 4-spored asci in each case, much larger numbers of the small unisexual ascospores should have been grown, so that several pairs of mycelia of opposite sex, each one with the luxuriant albinistic growth, were available for matings from which to select for 4-sporedness. Something of this sort was done incidentally when clons 22 and 34 were selected from among thirty-four back-cross haplonts of a second mating, $\{(507 \times 56.2)_{29} \times S_6\}_8 \times S_6$. They were of opposite sex, somewhat albinistic, and non-conidial. The basis for their selection aside from being non-conidial was that when grown together a great abundance

of ascocarps was obtained, a doubtful procedure when this was not the particular character desired.

The asci from this mating were mostly 4-spored; yet there was enough irregularity as indicated by the frequency with which one found asci with giant spores as well as asci with five or six spores to enable one to readily distinguish these ascocarps from those produced by typical *N. tetrasperma*. By continued selfing one could obtain bisexual non-conidial strains which would produce 4-spored asci very regularly.

Four 4-spored asci from the mating 22 × 34 were analyzed. Some of the characteristics of the mycelia in each case are given to indicate that the fertility of the parent combination is not always passed on to the offspring, and that the fluffy albinistic type of growth has not been perpetuated through the various back-crossings.

Ascus A. 1. Non-conidial, bisexual, slowly fertile at first, then prolific.
2. Non-conidial, bisexual, very fertile.
3. Non-conidial, bisexual, very fertile although ascocarps appeared slowly at first.
4. Non-conidial, bisexual, abundance of perithecia.

All of these four mycelia produced only a scanty aërial growth; most of the asci were good 4-spored types.

Ascus B. 1. Non-conidial, bisexual, very fertile.
2. Non-conidial, bisexual. Thought to be sterile at first but a few perithecia finally matured.
3. Spore did not germinate.
4. Non-conidial, bisexual, very fertile.

Ascus C. 1. Spore did not germinate.
2. Non-conidial, bisexual, very fertile.
3. Non-conidial, bisexual, slowly fertile. Asci fairly regularly 4-spored.
4. Non-conidial. No perithecia have developed in culture.

Ascus D. 1. Non-conidial, very weakly bisexual.
2. The same as no. 1.
3. Non-conidial, strongly bisexual.

Clon 2A, which is strongly bisexual, and 3C, which is rather

weakly bisexual, were among the so-called homothallic strains which were later hybridized directly with certain unisexual strains.

Twelve ascospores of normal size taken at random from the spore print on tube 22 × 34 gave non-conidial bisexual mycelia. All produced an abundance of perithecia except no. 3, which developed only a few after many days. The asci were mostly 4-spored. Clon 8 in this series was the only one which had anything resembling the fluffy albinistic type of aërial growth.

CROSSING HERMAPHRODITIC WITH HETEROTHALLIC RACES

The discovery that certain species of *Mucor* were heterothallic first led Blakeslee to attempt to obtain hybrids. The possibility of hybridizing species of ascomycetes was not seriously considered until very recently. No doubt it was deemed futile to try to cross species unless mycelia of opposite sex were developed. Although Thaxter had previously described several species of the Laboulbeniales as "dioecious," it was not until the life history of *Ascobolus magnificus* was studied (**4**) that any ascomycete was proved culturally to be heterothallic. Strangely enough the first hybrid ascomycetes produced were between species rather than between races of the same species. Crossing species of yeasts such as *Schizosaccharomyces octosporus*, where fertilization is assured by the fusion of two free bud cells, would not be a question of heterothallism versus homothallism. It would all depend on the efficacy of cross fertilization as compared with close inbreeding through self fertilization. The same would be true for forms like *Taphrina epiphylla*, which is known to be heterothallic (**13**).

Every nucleus in the mycelium of *Pyronema*, up to the time of the differentiation of the sex organs, carries the potentialities for complete sexual reproduction. The chances of getting such a species to hybridize would seem on first thought to be remote. It was only because *Neurospora tetrasperma* occasionally develops a small unisexual ascospore that the crossing of this species with heterothallic *N. sitophila* seemed worth trying. One may argue that *Pyronema* and *Neurospora tetrasperma* are not comparable in their homothallism because in the latter species there are two kinds of nuclei as to their sex. But so there are in *Pyro-*

nema after the differentiation of the two branching systems giving rise to oögonia and antheridia respectively.

Several examples were given in previous pages where a monosporous mycelium, which appeared for a long time as tested to be unisexual, finally gave rise to a few ascocarps. The ascospore producing such a mycelium must have originally contained one nucleus of each sex. Many examples are recorded in the literature on culturing ascomycetes of the effect of bacterial or other fungous contaminants accidentally or intentionally introduced into the culture. *Ascobolus Winteri* is homothallic. Yet if one inoculates a plate culture on opposite sides with transplants from the same monosporous culture two rows of ascocarps are formed along the line where the two mycelia meet (**5**). With such examples as these in mind when it was found that the weakly bisexual strains referred to above fruited abundantly with the one or the other tester strains S_1 and S_6, the question arose as to whether the tester strain may have been acting merely as a nutritive contaminant, not taking any part whatever in fertilization. On the other hand if the progeny from the ascocarps showed characters, certain ones of which were present only in the tester strain, this would prove a preference for cross fertilization to selfing, thus suggesting a way of hybridizing homothallic strains. Experiments planned to prove that this can be done will be described presently.

The origin of the strains mentioned previously and in connection with the matings to be described in succeeding paragraphs can be seen at a glance in the following tabulation.

S_6. A unisexual sex A haplont of *tetrasperma*.
S_1. A unisexual sex B haplont of *tetrasperma*.
209. $\{(sitophila \times tetrasperma)_1 \times S_1\}_9$ = sex A haplont.
507. $(209 \times S_1)_7$ = sex A haplont.
56.2. Non-conidial sex B haplont of *sitophila*.
Asci C′, G, L and M′. $\{(507 \times 56.2)_{29} \times S_6\}_8 \times S_6$.
1C′. Bisexual mycelium derived from spore no. 1 from ascus C′. The nuclei of only one sex carry +C factors for conidia.
1G, 2G. Bisexual mycelia derived from spores 1 and 2 from ascus G. All nuclei of both sexes carry the +C factors for conidia.
3G, 4G. Bisexual non-conidial mycelia from spores 3 and 4, ascus G.
5M′. Bisexual mycelium from spore no. 5, ascus M′. Only the sex A nuclei carry +C factors for conidia.
22. $[\{(507 \times 56.2)_{29} \times S_6\}_8 \times S_6]_{22}$ = Non-conidial sex A haplont.

34. Sex B haplont. Same origin as no. 22; also non-conidial.
Asci (4-spored) A, B, By, C. Asci from the mating 22 × 34.
1A, 2A, 2B, 2By, 3C, etc. Bisexual non-conidial mycelia from spores from asci A, B, By and C respectively.

The distribution of perithecia in a plate culture of *N. tetrasperma* inoculated on one side with strain S_1 and on the opposite side with strain S_6 is characteristic. The first fruit bodies begin to form where mycelium S_6 meets S_1. Others appear one by one back along the lines of growth of S_6 hyphae radiating from the point of inoculation. Ascocarps do not form on the area occupied by S_1 mycelium (PLATE 7, FIG. 17). This does not prove however that strain S_6 is female. When a culture of *Ascobolus magnificus* (**4**) is prepared in a similar manner one readily sees where the two mycelia come together, stop growing for a short time, then grow right on over so that there is a complete intermingling of the mycelia of both sexes. Finally ascocarps are found scattered here and there all over the culture. Should there be any such intermingling of hyphal elements in cultures where strains S_1 and S_6 of *N. tetrasperma* are grown from opposite sides it is certainly not readily detected. Inoculate a plate culture with strain S_1 alone, and, after the new mycelium has covered the plate, transfer to this plate a small block of agar taken from a similar culture containing only S_6. Ascocarps develop principally, if not exclusively, on the little block of S_6 agar. Reverse the process and transfer a little block containing S_1 mycelium to a plate culture of strain S_6 and no ascocarps form on the little block, but they do develop right beneath it and then spread out in the S_6 area. The area finally containing fruit bodies is usually not large however. These few experiments are presented to show certain peculiarities or cultural characteristics which have been found helpful in explaining the results obtained in other experiments.

The picture formed when a plate culture is inoculated on opposite sides with normal bisexual mycelium of *N. tetrasperma* in which every nucleus carries the +C factors for conidia is shown in PLATE 5, FIG. 1. Ascocarps first appear at the points of inoculation and then spread out in radiating lines. Instead of forming a dense line of perithecia where the two mycelia meet we have a zone comparatively sterile. This zone gradually

narrows as the culture ages. Yet one sees a certain antagonism which inhibits sexual reproduction although the two mycelia leave no area unoccupied.

A similar picture (PLATE 5, FIG. 2) is developed when the culture contains two plantings of 5M′ in which only sex A nuclei carry the +C factors for conidia. Although 5M′ is a hybrid segregate many of its asci are 4-spored. Compare the distribution of ascocarps in the two cultures just described with what one finds when either *N. tetrasperma* or 5M′ is grown opposite S_1, which is sex B and conidial (PLATE 5, FIGS. 4, 6). So far as one can see S_1 takes no part here in sexual reproduction, serving only to block the advance of the bisexual mycelia which are producing ascocarps right up to the line of meeting. When however strain S_6, which is sex A and less strongly conidial, is grown opposite either *N. tetrasperma* or 5M′, a somewhat different reaction occurs (PLATE 5, FIGS. 3, 5). Here quite a few fruit bodies are formed on the S_6 mycelium, many of them being distributed down along the lines of hyphal growth. Consider first the culture containing *tetrasperma* and S_6. Does S_6 take an active part in the sexual process, or are the ascocarps wholly the product of the bisexual *tetrasperma* mycelium which has now grown down over the S_6, perhaps because S_6 is less antagonistic to it than is S_1? It would be difficult to prove which is the correct answer because of the maze of fine hyphal branches growing in all directions in cultures of this age. Furthermore the ascocarps would be typical *tetrasperma* in either case because S_6 is merely unisexual sex A *tetrasperma*.

The solution of the problem would be scarcely less difficult in case of the culture where 5M′ is grown with S_6 (PLATE 5, FIG. 5) because the nuclei in 5M′ which carry the +C factors for conidia are of the same sex A as are those of mycelium S_6. It has been stated previously that S_6 was the back-cross parent in the origin of 5M′, so that it would be a long and difficult undertaking to prove by analysis whether the S_6 here was again the back-cross parent in the production of the ascocarps now formed on the area occupied by it in this culture.

When the non-conidial haplonts nos. 22 and 34 are mated in culture the asci formed are mostly 4-spored. Here also the 5- and

6-spored asci are numerous enough to enable one to distinguish the perithecia from those formed by *tetrasperma*. Plate cultures were prepared by inoculating one side with *tetrasperma* and the opposite side with either no. 22 or no. 34. Perithecia formed as usual on the area occupied by the *tetrasperma* mycelium, being most abundant around the point of inoculation, thinning out abruptly as this mycelium approached the one from the opposite side. In the meantime, many ascocarps had developed in a narrow zone along the line in each case where the two mycelia met (PLATE 6, FIGS. 7, 8). No. 34 is sex B; yet if we replace it with S_1, no such line of perithecia is formed, as previously noted (PLATE 5, FIG. 4). There can be no question that the hermaphroditic *tetrasperma* has hybridized directly with both no. 22 and no. 34 in the cultures figured. The fruit bodies are larger than are those formed on the *tetrasperma* area. There is more than enough irregularity in the number of spores in the asci to prove hybridity.

Reverse the process, and grow the hermaphroditic race 2A on one side and either S_6 or S_1 on the opposite side, and we find exactly the same picture formed (PLATE 6, FIGS. 9, 10). The reason is clear. S_6 and S_1 are *tetrasperma* with the sexes separated in two different mycelia, and 2A is simply the product of a mating or a union between 22 and 34.

Clon 3C is weakly bisexual. It has the same origin, however, as 2A. When 3C is grown opposite either S_6 or S_1, ascocarps form first along the line where the 3C meets the S_6 or the S_1, as the case may be. Spores are discharged from these ascocarps several days before fruit bodies on the 3C area are fully developed (PLATE 6, FIGS. 11, 12). The hybrid nature of the ascocarps along the lines of meeting of 3C and S_1 has been proved beyond question. They are fully twice as large as are those of 3C. Both series of perithecia have irregularity in the number of spores in their asci, so this feature could not be used as proof of the point in question. If strain S_1 has contributed nuclei which have entered into the fusion nuclei in the asci, some of the ascospores should produce mycelia with monilioid conidia. From among the ascocarps at the center of the line, two or three which had formed wholly beneath the surface of the agar were selected.

This was done not only to avoid the possibility of carrying over ascospores which might have been shot over from the 3C area, but also to lessen the chances of contamination by conidia from the S_1 area, which was the important thing.

Some of spores of the first sowing germinated without heating. Sixteen, judged by their small size to be unisexual, were transferred, and the remainder were given a severe heat treatment to kill the conidia present, which were now germinating rapidly. Many more ascospores were stimulated to grow. Forty-three were selected at random without regard to their size, and four isolated from one ascus were also included. The sixteen spores which had germinated without heating all gave rise to non-conidial mycelia. Only one was bisexual; the others were all of the same sex, A. All of the forty-three mycelia derived from the heated spores produced conidia in varying amounts. Thirty-three were bisexual and ten were unisexual, of which five were sex A and five were sex B. The production of conidia by so many of these single ascospore mycelia proves most conclusively that haplont S_1 had actually hybridized with the bisexual, totipotent race 3C. Mr. Carl C. Lindegren, who is especially interested in determining why some spores germinate without heating while others require severe heat treatment, has conducted some further experiments, analyzing individual asci from this same cross. He is including these results in a paper shortly to be sent to press. On the main question raised above, namely, the parentage of the ascocarps developed along the line where mycelia 3C and S_1 meet, his results furnish all the supporting evidence that could be desired for the conclusion that haplont S_1 is the sex B parent. Mycelium 3C contains nuclei of both sexes which unite in ascocarps arising from self fertilization. Given an opportunity where the two mycelia meet, cross fertilization with S_1 occurs. Haplont S_1 contributes the sex B nuclei and mycelium 3C only sex A nuclei.

It will be seen from the picture presented (PLATE 6, FIG. 11) that, in the plate culture in which S_6 was grown opposite 3C, ascocarps are formed along a rather broad zone where the two mycelia meet in much the same manner as in a culture of 3C with S_1. Other cultures of 3C + S_6 show a double line of fruit bodies. One can see the double line more distinctly in the reverse view

of the plate culture (PLATE 7, FIG. 13), which shows that many ascocarps here developed wholly beneath the surface of the agar. The fruit bodies on the S_6 side were very likely hybrids between 3C and S_6 and therefore some of the ascospores should give rise to mycelia with monilioid conidia like S_6. On the other hand, it might be that the ascocarps along the line on the 3C side were wholly the product of strain 3C alone, forming fruit bodies along the line where further growth was checked, or where the nutrient was being exhausted. In this case all of the ascospores should give rise to non-conidial mycelia. With the view of learning the meaning of this double row of fruit bodies, cultures were obtained from their ascospores. Two ascocarps which had developed beneath the surface of the medium at the point "X" on the S_6 side and two others "Y," from the 3C side, were selected for the test. The crushed mounts did not show any particular differences. In both sets the asci were mostly 4-spored, with some 5-spored asci characteristic of these back-cross hybrids mixed in with them. The cultures derived from spores taken from the "X" perithecia formed on the S_6 side of the zone will be noted first.

From among the spores which germinated without heating twenty-one were chosen at random without regard to their size. Twenty of their mycelia produced conidia, and only one was non-conidial. This was just the reverse of the case in the mating 3C × S_1 where the sixteen spores which germinated without heating were all non-conidial. One of the mycelia produced a few perithecia. The others were all tested against S_1 and S_6. Eleven finally developed perithecia when grown with S_1, reacting as sex A. The other nine did not produce any fruit bodies when grown with either S_1 or S_6 in two separate tests, illustrating again rather strong incompatibilities.

After removing some of the spores which germinated without heat, the plate was given the heat treatment. Practically all of the remaining spores germinated. Twenty-seven of their mycelia produced conidia and three did not. Twelve mycelia developed many ascocarps and were therefore bisexual. The other fifteen were all tested against both S_1 and S_3. Thirteen produced no ascocarps when grown with either one of these two

tester strains. It has been noted previously that certain mycelia of such hybrids produce perithecia only after many weeks in culture. This proves that they were really bisexual. No doubt something of this sort is operating here, because a larger proportion of the mycelia from ascocarps chosen more or less at random should have developed ascocarps readily. Much of the self- and cross-sterility exhibited in this series was very likely due to incompatibilities which should be made evident when other tester strains are employed. These tests were not carried further because the main point in question, namely, the nature of ascocarps along the line of the S_6 side of the culture, was settled. The hermaphroditic mycelium 3C does not produce monilioid conidia, and S_6 alone does not develop ascocarps. The conidial mycelia obtained from these ascospores are proof that 3C contributed the sex B nuclei and S_6 furnished the sex A nuclei which combined in the hybrid asci. It so happened that most of the ascospores selected at random gave conidial mycelia just as would be expected. No doubt an analysis of the spores from 5-spored asci would show that about half of the small unisexual spores would give rise to non-conidial mycelia.

Spores from the ascocarps "Y" from the 3C side of the culture 3C + S_6 which made the double line of ascocarps (PLATE 7, FIG. 13) were sowed in the usual way. Nineteen spores which germinated without heating were selected at random. Three of their mycelia were bisexual and the other sixteen were unisexual, sex A. Ten spores which required heat to induce germination were bisexual and one was of the type with weak sexuality. It reacted with neither S_1 nor S_6, the only strains it was tested against. The fact that all of the bisexual mycelia were non-conidial is the best possible proof that the ascocarps along the line on the 3C side were the products of this strain alone. The meeting of 3C and S_6 brought about a condition favoring production of ascocarps by the bisexual strain. The S_6 mycelium merely acted as an obstruction to further growth of the 3C mycelium. A colony of bacteria, some other fungus, or the side of the petri dish (PLATE 7, FIG. 14) might serve as well. With an equal opportunity for either self-fertilization or cross-fertilization, which method would prevail would depend on what strains are grown together.

Certain writers have interpreted the production of ascocarps along the line of meeting of two mycelia as proof of heterothallism. An analysis of the progeny from individual asci would show whether this were true or not.

The evidence presented certainly justifies the conclusion that certain hermaphroditic strains will hybridize directly with certain unisexual strains. The formation of large hybrid ascocarps along the line of meeting suggests the working out of a preference for cross-fertilization over selfing. Such hybridizing goes on equally well with either sex, S_6 and S_1, on the one hand, or no. 22 and no. 34 on the other.

CROSSING TWO HERMAPHRODITIC RACES

We have seen that neither of the hermaphroditic strains *tetrasperma* and 5M′ crosses with S_1 in a plate culture, while 1C′, 3C and 2A do so readily. With S_6, 2A acts much like 3C. Some cultures show two distinct lines of fruit bodies along the zone where the two mycelia meet. No doubt the ascocarps along the line on the 2A side are the product of 2A alone, while those on the S_6 side are hybrids between the bisexual totipotent 2A and the haplont S_6.

Several different bisexual non-conidial strains such as 3C, 2B, 2By, 3G, 4G and "42," whose asci are mostly 4-spored, have been developed. These strains have been grown in plate cultures opposite bisexual conidial strains such as straight *tetrasperma*, 1G and 2G, where all of the nuclei carry the +C factors for conidia. Each strain in every culture produced its own ascocarps and these ascocarps were variously distributed. In some cases the fruit bodies scattered irregularly along the line of meeting were clearly due to checking of growth. Distinct lines of ascocarps were formed in cultures of several different matings in such a way as to leave little doubt that cross-fertilization and hybridization had occurred between the two mycelia, each totipotent in itself (PLATE 7, FIG. 18).

N. tetrasperma formed such a line with clon 1C′ in plate cultures. Some of the fruit bodies were clearly pure *tetrasperma*. Their asci were very regularly 4-spored. Others showed odd-shaped over-sized ascospores and not a few 5-spored asci. It

would not always be easy to prove them hybrids, *tetrasperma* × 1C′, as distinct from ascocarps, the product of 1C′ itself. One of the two original nuclei included in spore 1C′ carried the +C factors for conidia. If, for example, this nucleus was of sex A, and clon 1C′ contributed the sex A nuclei and *tetrasperma* the sex B nuclei, then, besides numerous 4-spored asci, one would find quite a few with five or six spores. Furthermore, every ascospore, large or small, would give rise to a conidial mycelium. This would also be true for pure *tetrasperma*, but the percentage of 5-spored asci would be much smaller. Should clon 1C′ contribute its non-conidial sex B nuclei in a mating, and *tetrasperma* the sex A nuclei, then the resulting ascocarps could scarcely be distinguished by any test from those formed by 1C′ alone.

Another mating of two hermaphroditic clons the hybrid nature of whose ascocarps could be readily distinguished from those formed by either of the bisexual parents through self-fertilization would be such that in one mycelium only the sex A nuclei carry the +C factors for conidia while in the other mycelium the +C factors are carried only by the sex B nuclei or vice versa. The sex A nuclei in clon 5M′ are conidial, the sex B nuclei being non-conidial. The sex B nuclei in clon 3L are conidial and the sex A nuclei are non-conidial. Hybrid ascocarps between these two races would give ascospores which would all be either conidial or all non-conidial, depending on which parent furnished the sex A nuclei. No asci would have both kinds of spores. Asci from perithecia formed by either parent through selfing would always show segregation of the factors for conidia, so that some of the small spores would be conidial and some would be non-conidial.

Most of the matings between hermaphroditic strains were made in plate cultures on 4 per cent corn-meal agar. Such hard agar stimulates aërial hyphal growth, but often retards fruiting. The results of the preliminary experiments represent only one or two cultures of each pair grown together. Further work culturing from spores produced by the ascocarps formed along the line of meeting may show that what are now taken to be hybrids are merely products of self-fertilization by the one or the other strain. The following pairings of bisexual clons produced a line

of fruit bodies where the mycelia met, in addition to those produced by each one (*N. tetrasperma* is abbreviated "Tet"): Tet × 3C; Tet × 2B; Tet × 2By; Tet × 3C; Tet × 3G; Tet × 4G; 1G × 42; 2G × 1A.

No line of ascocarps was formed where the following bisexual mycelia met in plate culture: Tet × 5M′; Tet × Tet; 5M′ × 5M′; 2G × 2B; 2A × 2C; 2G × 3C; 2G × 4G; 1C′ × 2By; 2G × 42; 3C × 3C.

Practically the only place where crushed mounts would serve to indicate hybridity would be where pure *tetrasperma* was grown opposite a strain which formed asci with noticeable irregularities as to the number of spores in the asci and spore shapes and sizes. For example, three different types of ascocarps are readily distinguished in culture Tet × 2B. Crushed mounts of fruit bodies from the *tetrasperma* area show great uniformity and abundance of 4-spored asci. Those from the 2B area show irregularity. Ascocarps taken from the line of meeting where the two mycelia could hybridize show some irregularities such as might be expected of intermediates. Culturing mycelia obtained from the ascospores selected at random, while not altogether dependable, usually serves to settle the question. The simplest and the best way, in case of the cross Tet × 2B, would be to grow each spore from just one 5-spored ascus. If the five spores all produce conidial mycelia, the ascocarp originated from *tetrasperma* alone by selfing. If all five spores produce non-conidial mycelia, the ascocarp arose on mycelium 2B without crossing with *tetrasperma*.

This line of reasoning is based, of course, on the assumption that if two hermaphroditic races cross, one race contributes only sex A nuclei while the other race furnishes only sex B nuclei. Should, however, the perithecial primordia include one or more pairs of nuclei of both sexes from each race, complications would arise. With a nuclear fusion in the ascogenous cell, followed by a second fusion in the young ascus, various types of asci would be developed from which different kinds of haplont progeny would be segregated out. It is not inconceivable that cross-fertilization might be of such a nature that two nuclei of opposite sex from one race and only one nucleus from the other race enter the primordia. Some of the F_1 asci might contain the inheritance of

one parent only, and the other asci might be hybrids. At this writing the work has not reached a point where it is desirable to warrant positive conclusions as to the nature of the fruit bodies developed along the line of meeting such as shown in PLATE 7, FIG. 18.

The use of the term hybrid in connection with the experiments reported above has perhaps been a rather loose one. A perithecium has been referred to as a hybrid when it is meant, of course, that the asci within it are hybrid. The framework of an ascocarp is very likely purely gametophytic. It may be a mixture of such hyphal elements from both parents, or it may be formed from the hyphae of the one or the other parent mycelium without altering the nature of the asci within.

In their report on a re-investigation of *Sphaerotheca*, *Polystigma* and *Pyronema*, the Moreaus (**12**) have included the results of their cytological study of the three species of *Neurospora*. They state that the ascogenous cell contains at first several nuclei, all but one of which degenerate. The ascogenous hyphae then grow out from this uninucleate cell. The cells of the ascogenous hyphae are also uninucleate. The crosier or ascus hook arises from a stalk-cell which has only one nucleus. There is no antheridium or other structure which can be seen to function as such. The writer is not ready as yet to say whether this account of nuclear behavior in the origin of the ascogenous elements of *Neurospora* is correct or not. Leaving the subject of sex aside for the time being, there is still to be explained, on the basis of the facts presented by these authors, the very definite Mendelian inheritance of the albinistic non-conidial characters in matings such as have been described by the writer previously (**3**) and in the present paper.

If the crosier arises from a cell containing only one nucleus, as stated by the Moreaus, that nucleus must in some way have become heterozygous for the characters being considered. Was this through a previous nuclear fusion in the ascogenous cell similar to that described by Harper, Gwynne-Vaughan and others? If so, how did nuclei from two different mycelia get into that cell? If no antheridium is present, was it originally through an anastomosing of vegetative hyphae? The picture presented

(PLATE 7, FIG. 17) when clon S_1 is grown opposite clon S_6 is highly interesting. Such a striking pattern must have some significance. S_6 contains only sex A nuclei. Sex B nuclei, which can be had only from S_1, in some way have been brought to every point now showing a perithecium. Perhaps it will be through genetical studies of cultures like those shown in plates 5 to 7, supplemented by cytological work, that the questions regarding nuclear activities in sexual reproduction, which have been in heated dispute for the last thirty or forty years, will become better understood. *Neurospora sitophila* is world wide in its distribution. *N. crassa* can be found in tropical countries and wherever sugar cane is grown. *N. tetrasperma* can be obtained at least from a number of culture laboratories. All three species are readily cultured, and with some patience can be made to hybridize and produce fertile offspring. They are admirably suited, therefore, for further genetical studies.

SUMMARY

Further experiments in crossing typical conidial races of the *Monilia* bread mold, *Neurospora sitophila*, with albinistic non-conidial races of the species indicate that asci from such a mating are heterozygous for conidia, and that segregations of the factors for sex and the factors for conidia occur independently. Both sets may segregate in the first nuclear division in the ascus, both sets in the second division, or one set in the first and one set in the second. The eight spores may thus alternate in the ascus: two and two; four and four; or two, four, two, as to their sex or the factors for conidia which they contain.

The albinistic non-conidial race of *N. sitophila* was crossed with typical conidial *N. crassa* and fertile hybrids were obtained. The F_1 asci usually mature only two or three spores. Of the forty-four haplont clons studied, only six were strictly non-conidial. Five were albinistic intermediates producing a few conidia abnormally. Thirty-three were conidial, resembling the *crassa* parent in coloration. The albinistic non-conidial f_1 strains were back-crossed with the *crassa* parent. The hybrids obtained were very fertile, with little abortion of the ascospores. The asci and spores resemble more those of the *crassa* parent. The

few asci analyzed showed that in some cases the segregation of the factors for conidia was completed either in the first or the second nuclear division, so that the spores are disposed two and two; four and four; or two, four, two; as was proved to be the case for intraspecific crosses in *N. sitophila*. Some evidence was obtained indicating that in these back-cross asci segregation of the factors for conidia is occasionally not perfect, resulting in intermediate types with albinistic aërial growth, but with a few conidia rather abnormally formed.

Hybrids were obtained by crossing the albinistic non-conidial race of *N. sitophila*, which has 8-spored asci, with a weakly conidial, "scanty growth," 4-spored segregate 507, resembling *N. tetrasperma* as to its asci. The F_1 asci were 8-spored, but with much spore abortion after delimitation. Over 60 per cent of the f_1 haplonts grown from ascospores chosen at random were non-conidial. Some of these were fluffy albinistic types; others produced only a scanty aërial growth. Some haplonts produced highly colored conidia in far greater abundance than does the conidial 4-spored parent. When the non-conidial f_1 haplonts were crossed with S_6, pure *N. tetrasperma*, the asci developed in the fruit bodies from this mating contained variable numbers of spores, mostly from three to six. Mycelia obtained by growing the small unisexual spores showed still greater variation in the color and abundance of conidia produced. Again over 60 per cent of such haplonts did not produce conidia. Non-conidial segregate haplonts were again crossed with *N. tetrasperma* with the result that now many asci with four spores were produced. Spores from several asci were isolated in order and germinated. A number of the hermaphroditic mycelia obtained showed either weak sexuality or incompatibilities, some even approaching neutrals; otherwise segregations of the factors for sex and for conidia appeared to be Mendelian. Both nuclei in a bisexual spore from these hybrid asci may carry +C factors for conidia; one of the nuclei may be conidial and the other not; or both nuclei may be non-conidial, depending on the way the segregations of factors for sex and for conidia take place and the redistribution of the nuclei before the spores are cut out. When both nuclei carry the +C factors, the mycelium from that spore will develop

three kinds of conidia as regards their sex. If only one of the two nuclei in a bisexual spore carries the +C factors, then only one kind of conidia as to sex will be cut off.

Hermaphroditic, totipotent races were crossed with strictly unisexual races, and fertile hybrids were obtained as the result of a preference for cross-fertilization over selfing, the ascocarps forming along the line in each case where the two mycelia met in plate cultures. It was proved also that lines of ascocarps may be formed across a plate culture in which two such mycelia are grown, without any act of cross-fertilization entering into the phenomenon.

Some hermaphroditic races, such as pure *tetrasperma* and 5M′, which produce ascocarps freely on their own mycelia, when grown opposite each other in plate culture do not form lines of fruit bodies where the two mycelia meet. When certain other hermaphroditic strains, however, are grown opposite these races, such lines of ascocarps are formed where the two mycelia meet, and these fruit bodies are in addition to the ones produced by each mycelium over the area occupied by itself alone.

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EXPLANATION OF PLATES[1]

PLATE 1

Neurospora sitophila. Above. The albinistic non-conidial race of the common *Monilia* bread mold grown on white bread under a bell jar. The origin of this race is referred to in the text.

Below. The typical conidial race of the *Monilia* bread mold grown under the same conditions as the preceding. Note the masses of orange colored conidia which have fallen off. If the two races are mated in culture half of the progeny will be albinistic and half orange-colored, as shown in plate 2.

PLATE 2

Neurospora sitophila. Eight cultures showing the characteristics of the mycelia derived from the eight ascospores from an ascus produced as the result of crossing two races such as are shown in plate 1. Cultures 1 to 4 are albinistic and non-conidial; nos. 5 to 8 are typical conidial. Segregation of the factors for conidia occurred in the first nuclear division in the ascus. The sex of each mycelium is indicated by a colored circle on the tube, a green circle for sex A and a red circle for sex B. Segregation of the sex factors occurred in the second nuclear division.

PLATE 3

Neurospora sitophila. Above. Cultures representing the mycelia from the spores from a single ascus developed as a result of crossing two albinistic non-conidial strains like nos. 2 and 4 shown in plate 2, indicating that these races breed true for this albinistic non-conidial character. Sex factors segregated in second division.

Below. Cultures from the eight spores from a single ascus developed as a result of mating two typical conidial strains like strains nos. 6 and 8 shown in plate 2. These mycelia will always produce monilioid conidia in culture, and represent the common *Monilia sitophila* bread mold of older authors. Segregation of sex factors in the first division.

PLATE 4

Above. Nine cultures from ascospores of the mating $(56.2 \times 507)_{47} \times S_1$ chosen at random. Cultures 3, 4, 8 and 9 produced conidia; the others were non-conidial.

[1] Plates 1 to 4 are reproduced from photographs hand colored by Miss Fleda Griffith to whom the writer is much indebted.

Below. Ten cultures from spores of the mating $(56.2 \times 507)_{29} \times S_6$. Cultures 2, 6 and 11 produced conidia; all of the others were non-conidial. Such high coloration and profusion of conidia were not shown by either of the original parents.

PLATE 5

Fig. 1. Distribution of ascocarps in cultures inoculated on opposite sides with pure *tetrasperma*. Very few ascocarps develop along the line of meeting.

Fig. 2. Clon 5M′ is a hybrid segregate with many 4-spored asci. When planted on opposite sides of the culture the distribution of ascocarps is much the same as in the preceding. Compare with figure 16 in plate 7.

Fig. 3. Normal hermaphroditic *tetrasperma* grown opposite clon S_6, which is unisexual sex A *tetrasperma*. The ascocarps on the S_6 area are the products of cross-fertilization, the sex A nuclei being contributed by strain S_6 and the sex B nuclei by the bisexual *tetrasperma*.

Fig. 4. Same as the preceding, except that unisexual sex B clon S_1 replaces the strain S_6. Here no ascocarps are formed on the S_1 area. (See plate 7, fig. 17, for a picture of $S_6 \times S_1$ in culture.)

Fig. 5. Clon 5M′, when grown opposite S_6, produces many perithecia by self-fertilization, and also other perithecia by cross-fertilization with strain S_6.

Fig. 6. No ascocarps are formed on the area occupied by the mycelium S_1 in a culture with 5M′. Ascocarps are produced only by self-fertilization.

PLATE 6

Fig. 7. Hermaphroditic conidial *tetrasperma* grown opposite clon 22, a non-conidial sex A race. Above are ascocarps of *tetrasperma* by self-fertilization. Hybrid ascocarps are formed along the line where the two mycelia meet. Four of the nuclei in each ascus will be non-conidial, like those in no. 22, and four will carry the +C factors for conidia contributed by *tetrasperma* sex B nuclei.

Fig. 8. Same as preceding except that no. 34, sex B race, replaces no. 22. In this case *tetrasperma* sex A nuclei enter the cross and carry conidial factors. (See plate 7, fig. 15, for a culture of the pair 22×34.)

Fig. 9. Clon 2A, which is one of the progeny of 22×34, produces ascocarps abundantly by self-fertilization and also a line of hybrids with S_6.

Fig. 10. Clon $2A \times S_1$. A line of hybrid ascocarps between 2A and S_1, the same as in the preceding, except that here 2A contributes the sex A nuclei, and S_1 the sex B nuclei.

Fig. 11. Clon 3C is bisexual and a non-conidial offspring of 22×34. Its nuclei are somewhat incompatible, as only a few perithecia form by selfing. An indefinite zone of ascocarps where the two mycelia, 3C and S_6, meet.

Fig. 12. Clon $3C \times S_1$. The same as the preceding except the sexes taking part are reversed. Very few ascocarps form by selfing. Hybrid ascocarps formed at the line of meeting. At the left two rows of fruit bodies along the line of meeting.

PLATE 7

Fig. 13. Another culture of $3C \times S_6$. Note two rows of ascocarps where 3C meets S_6. The ascocarps in the upper row were proved to have been formed by 3C through selfing. Those in the lower row were hybrids $3C \times S_6$.

Fig. 14. Distribution of ascocarps when 3C is grown alone.

Fig. 15. Unisexual races 22 and 34 mated. Ascocarps formed in characteristic pattern on the area occupied by 22 which is sex A. Compare with fig. 17.

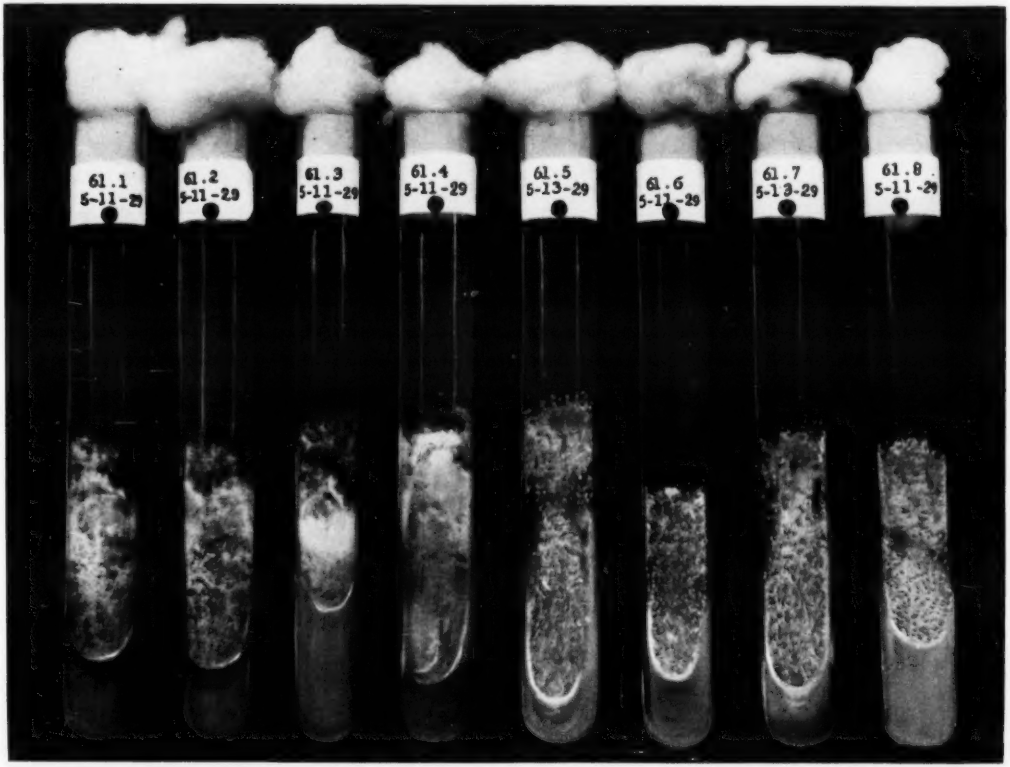
Fig. 16. Two hermaphroditic races, *tetrasperma* and 5M′, each self-fertile. No line of ascocarps along the line of meeting.

Fig. 17. Two unisexual strains S_1 and S_6 of *tetrasperma* species, grown together, form ascocarps only on the S_6, sex A, side.

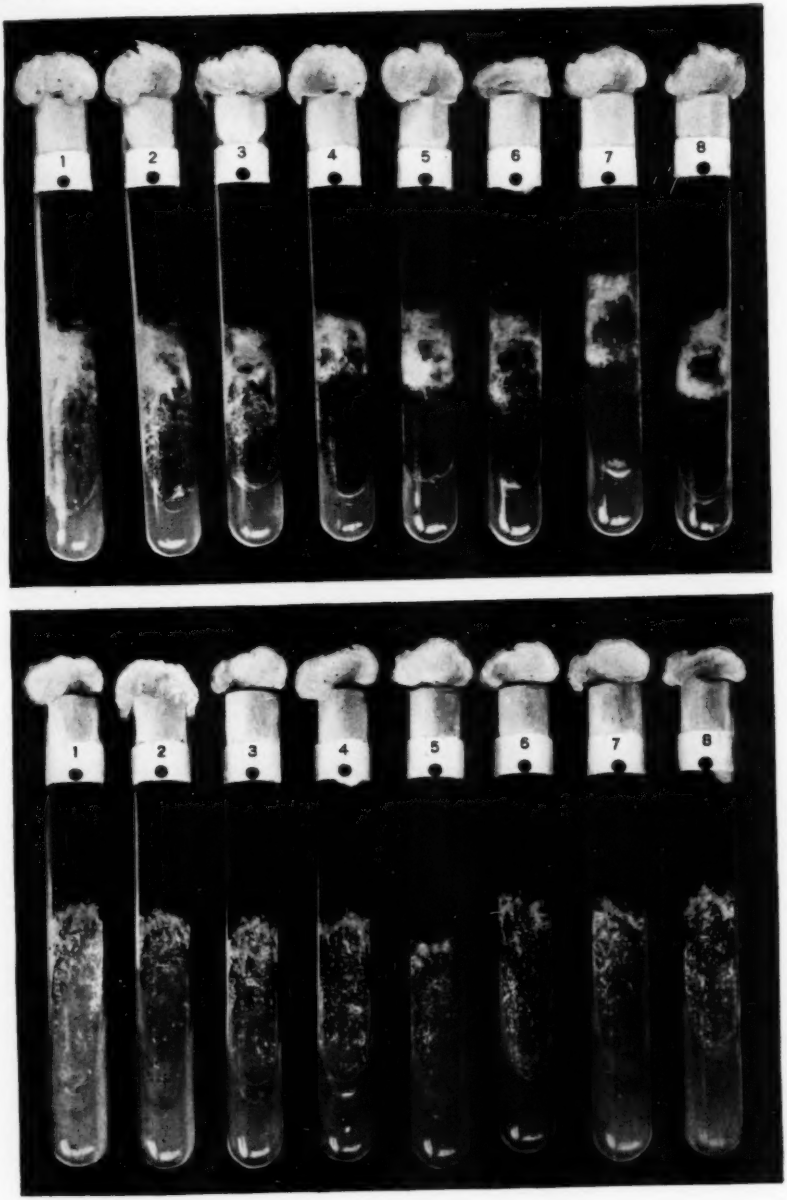
Fig. 18. Two totipotent hermaphroditic races, 2G and 1A, cross and produce a line of large ascocarps along the line of meeting.

NOTE

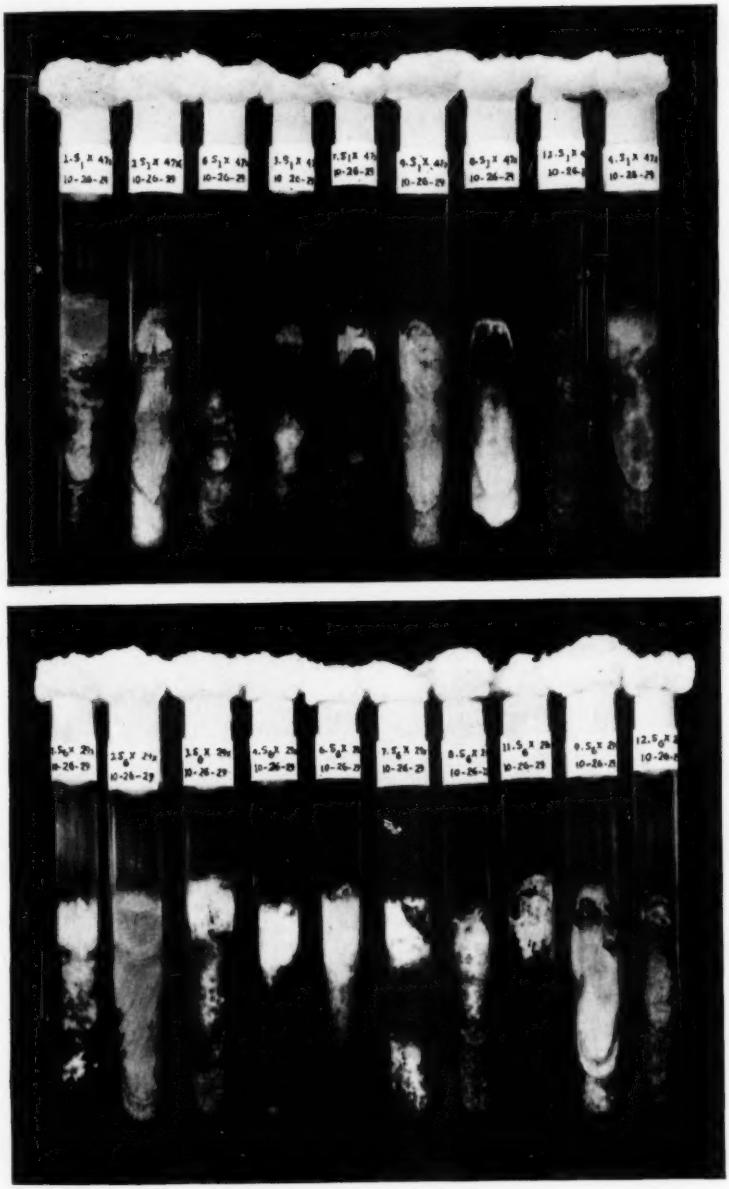
In this paper the terms hermaphroditic, homothallic, haplomonoecious and bisexual are all used in the same sense to indicate a mycelium having two kinds of nuclei as to their sex, or whatever it is here that corresponds to sex. Kniep's expression shortened to "mikto-haplo-monœcious" might be preferable.



SEGREGATIONS IN NEUROSPORA SITOPHILA



ALBINISTIC AND TYPICAL NEUROSPORA SITOPHILA



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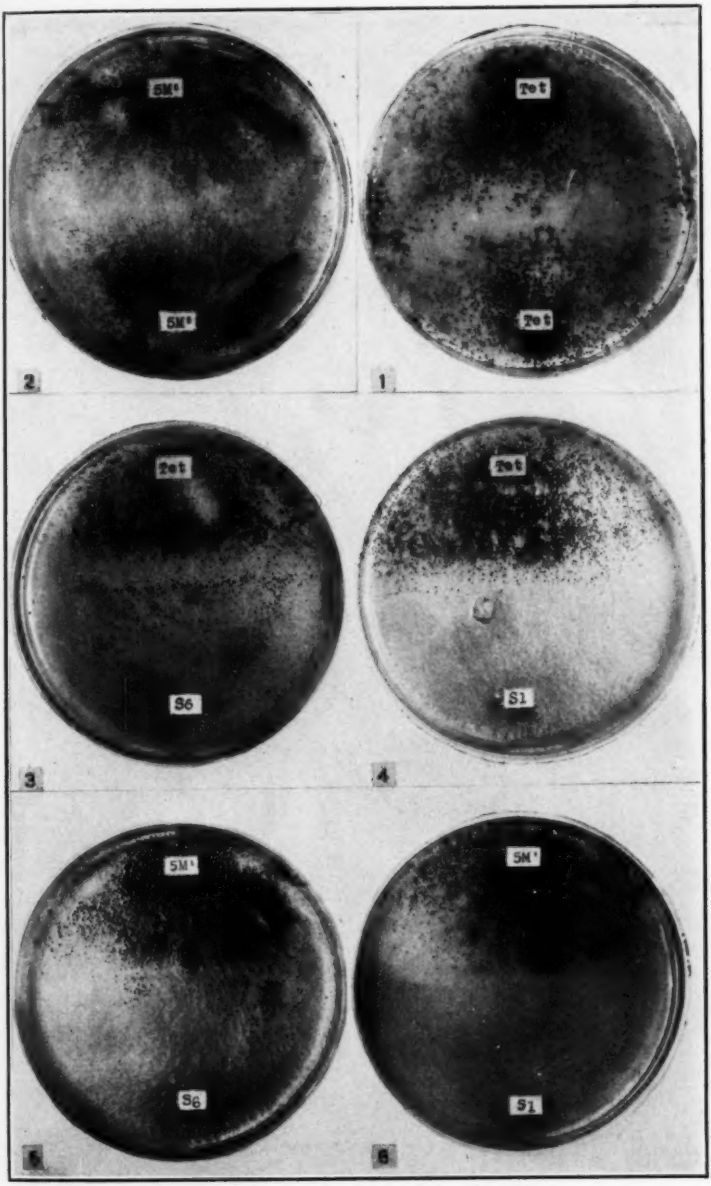
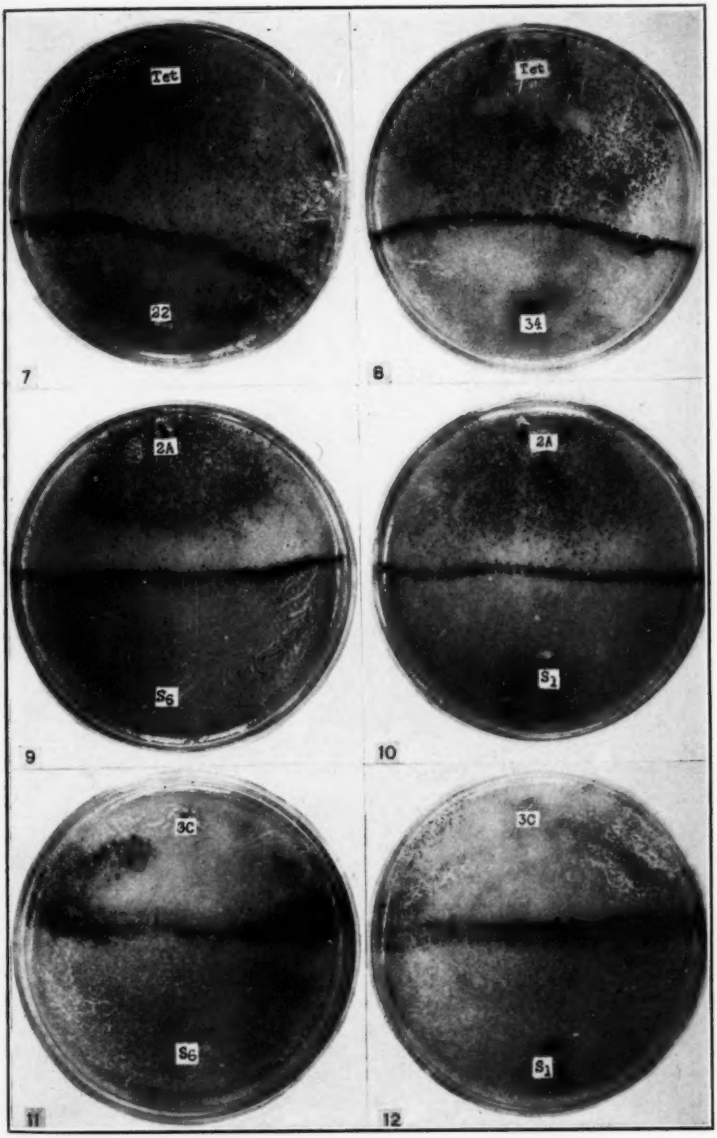


PAIRED CULTURES OF NEUROSPORA STRAINS

7

9

11



MATINGS OF NEUROSPORA HYBRIDS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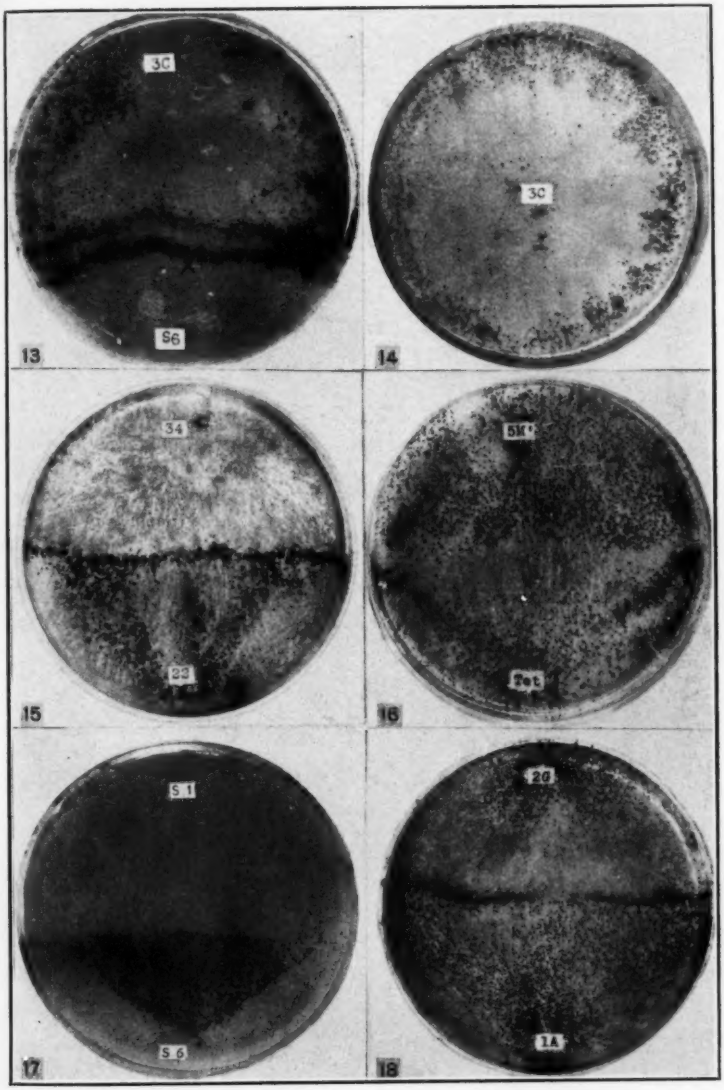
15
17



MATINGS OF NEUROSPORA HYBRIDS

STUDIES ON ASCOIDEA RUBESCENS
I. HISTORY AND DEVELOPMENT [1]

LEVA B. WALKER

(WITH 5 TEXT FIGURES)

There are few groups of fungi concerning which students of fungi are more curious than those commonly included in the Hemiascales. This curiosity exists because these forms show characteristics of both Ascomycetes and Phycomycetes but differ widely from typical forms in either group. Most of the forms placed in the Hemiascales are rarely found. Workers who study them find it difficult to trace satisfactorily their life histories. This has been especially true of *Ascoidea*.

HISTORICAL

The genus *Ascoidea* was established for *A. rubescens* by Brefeld and Lindau. The fungus was collected by Lindau during September and October growing in the sap flowing from felled beeches in the royal forest at Wolbeck. Brefeld (**1**) studied the fungus and in 1891 gave a comprehensive description of its gross morphology. Popta (**4**) carried further these studies and added observations upon its nuclear condition. These two papers have formed the basis of our general knowledge of the genus. The fungus has a coarse, branching mycelium which is abundantly septate at all times. On this mycelium abundant conidia and sporangia [2] develop. The sporangia which freely proliferate contain many small, walled spores imbedded in a granular, oily slime. The spores which are discharged from the sporangium at maturity have a characteristic hat-shape. Brefeld says they are formed in twos, but this is denied by Popta (**4**). Popta (**4**) found a multinucleate condition constantly present in vegetative and reproductive structures. Thus we have a fungus with a

[1] Cytological observations will be taken up in a second paper.

[2] The term, sporangia, used by Brefeld for these structures is continued but its use does not imply a morphological significance.

mycelium resembling that of Ascomycetes, forming sporangia in a Phycomycetous manner, but containing walled spores imbedded in a conspicuous slime that has at least a resemblance to the epiplasm of the Ascomycetes.

A second species of *Ascoidea*, *A. saprolegnioides*, was described by Holtermann (**2**) from materials collected from slime fluxes on various trees in Java. He distinguished this species from *A. rubescens* by the facts that (*a*) the proliferating growth forms one or more cells before forming a new sporangium and that (*b*) the sporangiospores lack the hat-shape.

No other papers dealing with *Ascoidea* seemingly appeared up to 1926 when Lohwag (**3**) published an extensive paper upon the homologies of the conidia of *Ascoidea*. This paper is apparently based upon the examination of mounts made by Schiffner from materials that the latter had found on maple trees in the Proter of Vienna. It contains little new information. However, Lohwag concludes that *Ascoidea* is in every respect a Phycomycete.

The last and only other paper is a brief summary of results obtained by Varitchak (**5**), a student of Dangeard. He describes the occurrence of two specially differentiated nuclei in the sporangium. These differentiated nuclei fuse but all other nuclei originally in the sporangium degenerate. The nuclei for the spores come from the repeated divisions of the fusion nucleus in a manner very similar to that described for *Dipodascus albidus*. He, therefore, concludes that *Ascoidea* is an Ascomycete. Thus the two latest workers dealing with the fungus come to absolutely contradictory conclusions as to the nuclear behavior in the sporangia and as to the relationships of *Ascoidea*.

Apparently the four collections of *Ascoidea* mentioned are the only ones recorded. No record of the occurrence of the genus in North America has been found. For this reason the author's collection of this fungus, August 8, 1927, from a slime flux on an elm tree at Ithaca, N. Y., becomes of especial interest. In spite of the fact that the fungus is unreported it seems probable that it is not uncommon in this country. During the fall of the same year many collections from similar slime fluxes on elm in the vicinity of Lincoln, Nebraska, showed remnants of *Ascoidea*

which had evidently been killed by the extremely hot, dry weather that had prevailed during the early part of September. For similar reasons none was found here during the summer and fall of 1928, *i.e.* there were no slime fluxes in which it could grow. The early spring of 1929 was damp and cool and slime fluxes began to develop upon elms, but as *Ascoidea* had always been found in late summer no examinations were made. Later an examination of these slime fluxes, then partially dried, showed the fungus had again been present but had been killed by heat and drought. Proliferations up to seven were often observed on such hyphal remnants. Occasionally one or more typical hat-shaped spores were found that had failed to escape from the sporangium and had been held between the wall of its sporangium and that of the proliferating sporangium. Typical conidia were also observed. All attempts to secure cultures from Lincoln collections were unsuccessful. The mycelium seemed dead and the spores failed to germinate.[3]

MATERIALS

The slime flux from which *Ascoidea* was collected for these studies occurred on the south side of a large elm tree situated at the foot of the "Forest Home path" leading down from the campus of the New York State College of Agriculture at Cornell University to the highway southeast of Beebe Lake.[4] The

[3] On June 11, 1930, just after the manuscript of this paper had been forwarded to the publisher, an abundance of very luxuriously developed *Ascoidea* was found in slime fluxes on elm trees near the College of Agriculture at Lincoln, Nebraska. May and June, up to that date, had been unusually cool and damp. The slime fluxes had developed from imperfectly healed pruning or other injuries on the trunks of the trees. In most cases the growth resembled in appearance the original collection. On several trees, especially on pruning injuries, the *Ascoidea* developed in definite mats 3–4 cm. broad, 7–8 cm. long, and about 1 cm. thick. These mats had evidently developed radiately. Actively growing hyphae, conidia, and sporangia were largely limited to surface areas. Hyphae and sporangia were commonly so definitely oriented and they gave the appearance of a vaguely defined hymenium. In several cases *Fusarium* was found growing with the *Ascoidea*.

[4] Dr. D. S. Welch, Cornell University, said that the tree had been injured about five years previously by two ax strokes. The upper of the two wounds healed normally but the lower, about four feet above the ground, developed the slime flux.

fungus continued its growth during the remainder of August, which was unusually cool and dry. During most of that period the area covered by the sap flow extended 12–18 inches below the top of the wound but refuse on the surface of the tree indicated that at times it must have extended almost to the base of the tree and covered a wedge-shaped area 4–6 inches across at the lower end. The flow of sap seemed to be confined to a strip a few inches in length at the top of the slime flux and to come from a rift in the bark hardly a half inch wide at the widest part. The bark below showed a continuation of the rift down to within about three inches of the ground. The bark along this rift, which seemingly showed remnants of slime flux on its surface, was much rotted and filled with all sorts of microscopic animals. *Ascoidea* was confined to a few inches at the top of the slime flux and the youngest and most vigorous growth was found in the upper half inch. The young growth was of a much lighter color than that below. The slime flux occupied by *Ascoidea* looked about the same as that lower down on the tree but when touched the part containing *Ascoidea* was readily seen to be made up of very coarse hyphae having much the texture and appearance, except for color, of a stunted growth of *Cladophora*. This filamentous, matted part of the slime flux gradually merged into the lower part of the slime flux which was an amorphous, granular slime. Mixed with *Ascoidea* in the upper part and filling the granular slime below were found myriads of microscopic animals, larvae, nematodes, etc., as well as yeasts and bacteria. No other filamentous fungus was observed in the slime nor did any develop in isolation cultures made from the *Ascoidea*.

The day following the finding of *Ascoidea* in the slime flux practically all of the fungus was removed from the tree. Isolation cultures were made, materials fixed in various solutions for sectioning, parts preserved in formal-acetic-alcohol and parts dried for herbarium purposes.[5] The fungus continued its development and two subsequent collections were made during August but the flux appeared to be drying when last observed by the writer near the end of August. It dried soon afterward,

[5] Specimens have been deposited in the herbaria of Cornell University, Harvard University, the University of Nebraska, the New York Botanical Garden, and the Missouri Botanical Garden.

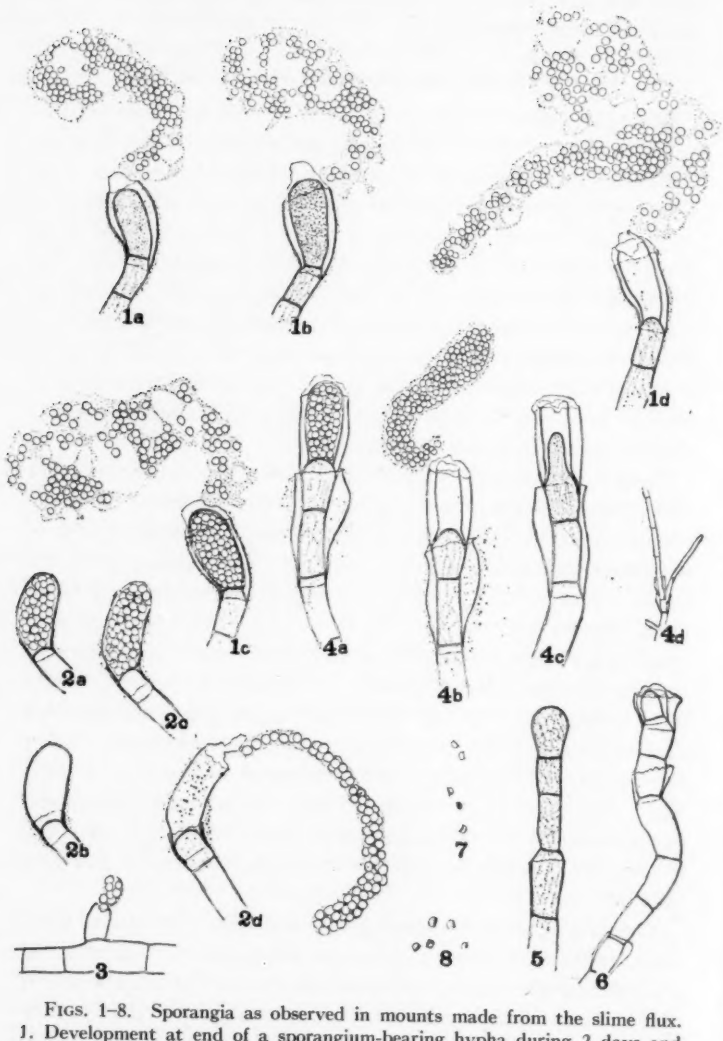
according to reports, and the development of *Ascoidea* in it has not since been observed.

OBSERVATIONS ON ASCOIDEA FROM SLIME FLUX

Materials taken directly from the slime flux were mounted in water in hanging drops. The mycelium consists, as described by earlier workers, of coarse, septate, branching hyphae (FIG. 9) in which the protoplasm shows conspicuous and characteristic longitudinal meshes (FIGS. 1–5, 10, 13). On the ends of some branches sporangia (FIGS. 1–6) might be present or develop while on others conidia (FIGS. 10–13) were found. Growth in hanging drops would usually continue about a week, gradually decreasing unless additional nutrients were added. When some nutrient was added very rapid growth would be resumed for a day or two but bacteria would develop so abundantly as to destroy the culture after that time.

Sporangia.—The development and discharge of many sporangia were followed in these water hanging drops. A few of these are shown in figures 1–6. The sizes and shapes of the sporangia were extremely variable. The most common type is shown in figures 1*c*, 2*a*, and 4*a*. Such sporangia developed on the ends of hyphae which widen slightly toward their tips (FIG. 4*a*). Smaller sporangia occurred and were usually observed as the nutritive supply became reduced. Many sporangia also arose laterally from hyphae (FIG. 3). In most of the sporangia the spores were so numerous that the number could scarcely be estimated, and in the smaller sporangia the numbers were extremely variable. The smallest sporangium observed (FIG. 3) contained eight spores. Successively larger sporangia contained 11, 16, 23, 84, etc., spores, the number varying according to the size of the sporangium.

The development of a sporangium usually required about three days. Figure 1*a* shows a sporangium about a day old, which is proliferating through an old sporangium, and the mass of spores that had been discharged from the old sporangium. At this time the young sporangium appears as a cell densely filled with highly refractive protoplasm. Figure 1*b* shows the same sporangium 16 hours later. The sporangium has enlarged and the protoplasm



FIGS. 1–8. Sporangia as observed in mounts made from the slime flux. 1. Development at end of a sporangium-bearing hypha during 2 days and changes in discharged spore masses: 1*a*, a young sporangium proliferating through an empty one and the discharged spore mass 4 p.m.; 1*b*, beginning of spore formation in the developing sporangium and increase in vacuolization

has taken on a foamy consistency which characteristically precedes spore formation. During the next 24 hours the spores became rounded and looked mature (FIG. 1*c*). The actual discharge was not observed in this case but figure 1*d* shows its appearance 6 hours later.

The details of a discharge were probably most clearly observed in the sporangium shown in figures 2*a*–2*d*. The first figure (2*a*) shows a mature sporangium that had proliferated through either a very small sporangium, or more probably a broken cell. The wall of a sporangium is made up of three layers, a thin outer, a thick middle, and a thin inner layer. The outer layer is evidently somewhat gelatinous and in mounts made from the slime flux always contained many minute granules of debris. Where present these granules have been shown by stippling. (These granules are absent around sporangia grown in pure cultures.) In figure 2*b* the rupture of the outer layer of the wall near the tip of the sporangium may be seen. It occurred 45 minutes after the condition shown in "2*a*" and was easily seen because of the granules in the outer slime. The ruptured layer is seen on each side near the tip. It was 4¼ hours later that the thick middle wall of the sporangium ruptured (FIG. 2*c*) and for two minutes the bulging spore mass was held by the delicate elastic third and innermost layer of the sporangium wall. When this inner wall gave way, in this case and all other cases observed, the spore mass rushed out rapidly at first, more slowly later, into the surrounding water (FIG. 2*d*). The spores emerging during the latter part of the discharge came out in almost single file. The various phenomena recorded here were repeatedly observed on various spo-

of spore mass 8 a.m. the next day; 1*c*, continued development 8 a.m. the second day; 1*d*, appearance after discharge of second sporangium 2 p.m. of the second day. 2. Details of discharge of sporangium: 2*a*, mature sporangum 12 m.; 2*b*, rupture of outer wall near apex (content not shown) 12:45 p.m.; 2*c*, rupture of thick middle wall 5 p.m.; 2*d*, rupture of inner wall and discharge of spore-mass as it appeared 2 minutes later. 3. A small lateral sporangium. 4. Development at end of a sporangium-bearing hypha: 4*a*, afternoon; 4*b*, 9 a.m. following day (transferred to bouillon and position changed); 4*c* and 4*d*, vegetative growth resulting. 5. Protoplasm in a hypha, the terminal cell probably a young sporangium. 6. Series of small empty sporangia through which proliferations had occurred. 7. Spores taken directly from slime. 8. Spores after standing in bouillon. 4*d* × 39; others × 200.

rangia but not enough were watched continuously to be sure whether the lengths of periods between the rupturing of the three layers in the wall were typical or not. It seems these periods may be very variable. Discharged masses from large sporangia were usually the shape of a curved club, while those from smaller sporangia were more globular. The time occupied by a discharge was only a few seconds. A discharge from a sporangium of *Ascoidea* appears much like the discharge of zoöspores from the sporangia of water moulds, such as *Achlya*, except for the presence of the densely granular slime between the spores. All of the spores were usually discharged in one mass which at the end of the discharge lay at a little distance from the mouth of the sporangium. Occasionally, however, a few spores became separated from the discharging mass and failed to get out of the sporangium. Such spores seemingly never escape from the sporangium and remain permanently between their old sporangium wall and the growth proliferating through it.

On the sporangium-bearing tip, shown in figure 1, the new sporangium was formed within the old one but in a majority of cases observed the proliferating hypha formed one or more vegetative cells before forming a second sporangium as shown in figures 4–6. Figure 4*a* shows one nearly mature sporangium and two empty ones. After the discharge of the first sporangium two cells were formed before the formation of the second sporangium while the third was formed directly within the second. After the discharge of the third sporangium the specimen was transferred to bouillon and the position of the sporangium changed. The nutrient medium caused a return to vegetative development with the results shown in figure 4*c*, 4*d*.

Figure 5 shows three vegetative cells and what is probably a primordium of a sporangium. Such broadened terminal cells with dense protoplasm and rounded vacuoles usually develop into sporangia but in some cases vegetative growth is resumed. Other workers have found it impossible to distinguish sporangium-bearing from conidium-bearing hyphal tips but in this material sporangium-bearing hyphae were broader than others and toward the apical end usually increased slightly in diameter. In many cases the cell just below the sporangium was sufficiently different

from the other vegetative cells to remind one of the suffulatory cells associated with oögonia in *Oedogonium*.

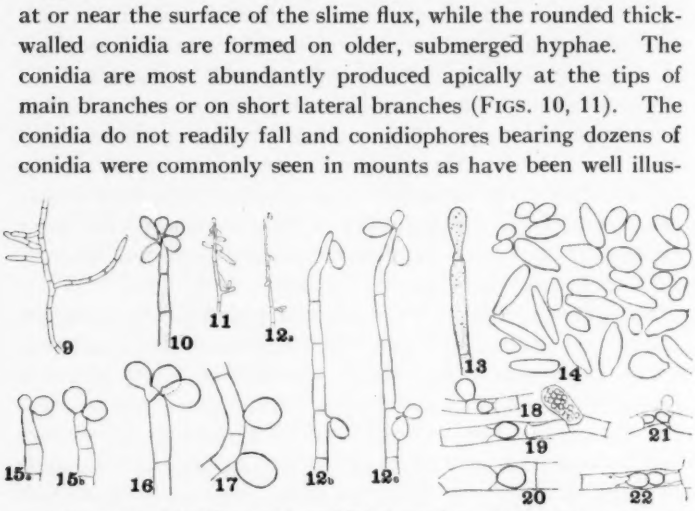
The spore mass as discharged from the sporangium consists of spores imbedded in a homogeneous, oily slime (FIGS. 2*d*, 3, 4*b*). Within a few hours vacuoles begin to appear in the slime. The vacuoles increase in number and size apparently by the absorption of water and thus gradually the spore mass is enlarged and the spores separated. Often the vacuoles become so large that they break open, leaving peculiar points and circular depressions in the outer part of the slime. These changes are illustrated in the spore masses shown in figure 1*a*–1*d*. The spore mass to the left in figure 1*d* shows the beginning of the vacuoles and the spore masses in figure 1*a*–1*c* illustrate the successive changes during 2 days in the same spore mass.

The spores as they lie in a spore mass appear as small rounded bodies of quite uniform size. When critically examined they appear as in figure 7, flattened on one side and having a hyaline projecting rim around the flattened side. These spores, commonly known as "hat-shaped," are characteristic of *A. rubescens* and a number of other Hemiascaceous fungi such as *Endomyces fibuliger*, *E. decipiens*, *Willia anomala*. Spores in bouillon or water enlarged somewhat (FIG. 8) but did not germinate. In young spore masses the spores are held so firmly in the slime that it is almost impossible to separate them but as the slime becomes more and more vacuolate it is often possible to separate them into smaller groups. No germinations of sporangiospores were secured from materials secured directly from the slime flux.

Microchemical tests show that most of the vegetative cells are densely filled with glycogen and many also contain oil. Young sporangia contain much glycogen and protein while mature sporangia contain little if any glycogen. No glycogen could be detected in discharged spore masses. The slime around the spores is an oily substance containing many granules, the nature of which could not be determined. They probably represent waste materials.

Conidia.—Conidia were abundant on all materials examined but no detailed studies have been made of them. Three types of conidia have been recognized by previous workers: cylindrical;

oval; and rounded, thick-walled, resting conidia. Although the three types are easily recognizable, there are such complete intergradations between types that such a classification seems useless. The oval and cylindrical types are commonly developed at or near the surface of the slime flux, while the rounded thick-walled conidia are formed on older, submerged hyphae. The conidia are most abundantly produced apically at the tips of main branches or on short lateral branches (FIGS. 10, 11). The conidia do not readily fall and conidiophores bearing dozens of conidia were commonly seen in mounts as have been well illus-



FIGS. 9–22. Mycelium and conidial formation. 9. Typical mycelium from slime flux. 10–13. Formation of conidia on younger hyphae. In 10 protoplasmic structure is indicated. 11. Variations in size and shape of conidia still adhering to conidiophore. 12. Development of conidia in hanging drop: 12*a*, conidium-bearing hypha; 12*b*, tip of same hypha enlarged; 12*c*, same tip 2 days later. 13. Attachment of a young conidium. 14. Variations in size and shape of conidia seen in a single field of the microscope in a 10 day old culture. 15–22. Development of conidia on older hyphae: 15, globular conidia on end of hypha; "b" 16 h. after "a"; 16 and 17, thick-walled conidia on old hyphae, developed apically in 16 and laterally in 17; 18–22, development of thick-walled conidia internally and laterally on old hyphae. Figs. 9, 11, 12*a* × 39; 10 × 200; all others × 215.

trated by Brefeld (1). The conidia are formed on the ends of hyphae that continue at once apical growth, pushing the conidia aside, and proceeding to form other conidia. Figure 12*a* shows a young branch bearing oval conidia. Its tip highly magnified may be seen in figure 12*b*, while 12*c* shows its appearance two days later in a hanging drop. Characteristically there is a narrowing of the spore just above the point of attachment, so

that a sort of stalk is formed at the basal end as shown in figure 13. The development of globular conidia is shown in figure 15*a*, 15*b*, the second drawing showing development 16 hours later. Another case is shown in figure 16. Such conidia have heavier walls and are formed after active growth has ceased.

Conidia are not only formed on the tips of branches but may be formed laterally (FIG. 17) or internally (FIGS. 18–22) from older, submerged hyphae. Such conidia are usually heavy walled and often the wall is somewhat colored. So far as observed they are of the rounded, resting type. The drawings for illustrating this type of development were largely made from cultures of *Ascoidea* but the same types were abundantly present in materials taken from the slime flux. Variations in the size and shape of detached conidia observed in a single field of the microscope are shown in figure 14.

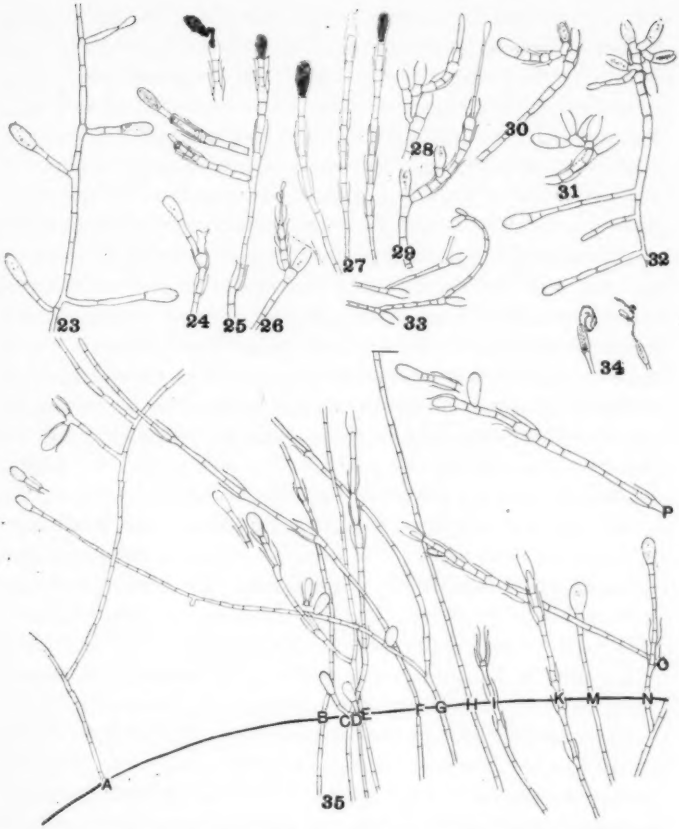
CULTURES

Seventeen pure cultures of *Ascoidea* were secured from the original materials. Some of these were from single conidia, others represent growth from several conidia and still others were secured from mycelial fragments. Growth in all cultures was similar. Because of the difficulties encountered in securing the germination of sporangiospores no cultures have been secured from this source. These cultures have been maintained and have furnished materials for the further studies here reported. Vegetative growth is good on all media tried but best in liquid media. The most satisfactory cultures have been on elm twigs nearly submerged in nutrient solutions. In these the fungus could grow over the wet surface of the twig, securing at all times ample moisture and exposure to the air. Of the many nutrient media tried the most satisfactory has been 0.3 per cent beef extract + 3 per cent dextrose in an elm twig decoction.

All cultures formed a multitude of cylindrical and oval conidia wherever actively growing mycelia reached the surface. The conidia were formed either in the air or in liquid near the surface. Thick-walled, globular conidia were formed in older cultures on submerged hyphae. If the entire culture was submerged no other reproductive structures appeared. Rarely were sporangia developed and when produced were usually quite abnormal.

They only formed in old, depleted cultures. In some cases normal proliferations up to seven were observed. Often the spores appeared to have been neither normally developed nor discharged, as the sporangia contained remnants of spores in a surrounding slime. If the development and discharge were only slightly abnormal so that most of the contents escaped the sporangium, proliferations took place normally as shown in figure 25. If very abnormal, a true sympodial development resulted. Figure 26 shows a commonly observed condition where the terminal sporangium appeared blighted and a branch just below it had continued the sporangial development, forming and emptying 4 sporangia and finally proceeding to the formation of conidia. The extreme of this type of development is shown in figures 28–33. The most plausible explanation for this variation is that lack of moisture prevented the normal development of the sporangium and growth was resumed below. The development of normal appearing sporangia below the aborted ones, as shown in figure 32, would suggest this. Similar development was repeatedly observed in slide cultures, as will be described later, that had become too dry. The presence of these sympodially developed sporangia adds weight to Lohwag's (**3**) contention that proliferations are modifications of the sympodial type of development as in this case the sporangia and conidia follow the same developmental sequence. All who have studied the fungus have held that conidia and sporangia are modifications of the same structure.

Many types of cultures were tried in the vain hope that an abundant development of sporangia might be secured. Brefeld (**1**) and Popta (**4**) seemed to have had little if any more trouble in securing abundant sporangia in old cultures than conidia in young cultures. These authors do not give any details as to how the cultures were handled. Various media, and changes from high nutrition to low nutrition, etc., were tried but in no case were sporangia ever developed sufficiently in pure cultures to supply materials for sporangial studies which were most desired. The first real success was observed when reëxamining a mount made three days previously from an elm twig culture that showed a few sporangia. The mount had been placed in a



FIGS. 23–35. Development of sporangia in cultures. 23. Hypha bearing 4 sporangia and one conidium developed beyond the edge of the cover glass in a mount held in a moist chamber for 2 days. 24–27. Commonly observed types of proliferations. 24. New terminal branch developed after one has blighted. 25. Left, proliferations through an incompletely emptied sporangium. (The insets show development one day later; the discharge of the terminal sporangium is somewhat abnormal.) 26. Aborted terminal sporangium and development of new sporangia below; the last proliferating hypha having developed conidia. 27. Three hyphae showing the most common types of proliferation and development of sporangia. 28–33. Variations in development of sporangia observed in old cultures, 32, showing a combination of normal and abnormal types of development. 34. Abnormal discharges of spores in long worm-like threads as described by Brefeld. 35. Development of sporangia at edge of cover-glass (heavy line). All insets show development 2 days later. Discharged spore masses were omitted. All × 50.

Petri dish on wet filter paper, hoping that more sporangia might develop. The original sporangia had matured normally and discharged their spores but no more had developed under the cover glass. The mount was about to be destroyed when it was observed that on a few hyphae, that had extended beyond the cover glass, over 20 young sporangia had developed. Figure 23 shows a part of one of these hyphae. During the next few days these sporangia matured, discharged their spores, proliferated, etc., in an entirely normal manner. Other mounts were made from the same culture tube and behaved similarly but mounts from other media and other cultures behaved variously and diverse results were secured in each series of experiments. In a long series of experiments abundant sporangia were secured at one or more times from all cultures used but no conditions were found that could be manipulated so as to secure uniform results. In these experiments mounts in nutrient media and in water, mycelium in thin films of water on slides held in moist chambers, and mycelium in thin films of water in Petri dishes and in hanging drop cultures were used. The most dependable development of sporangia was secured when masses of actively growing mycelia from a culture tube were: (*a*) washed in several changes of water; (*b*) let stand in thin films of water in a Petri dish for several days; (*c*) mounted in abundant water so that some mycelium was near or beyond the edge of the cover glass and placed in a moist chamber; and (*d*) let dry daily for several days till all extra water at the edge of the cover was gone and then rewatered. By this method 100 per cent of mounts in some series showed abundant sporangial development while in others almost no sporangia appeared. This variation may have been due to the age and vigor of the original culture, to the degree of drying given the mounts, to inherent tendencies, or to a combination of these factors. Old depleted mycelium exposed either in thin films of water in Petri dishes or at the edge of cover glasses in moist chambers usually developed sporangia.

The formation of sporangia almost directly from germinating conidia (FIGS. 53–57) and mycelial fragments (FIG. 58) has been secured repeatedly under quite different conditions. In each case mycelium from a culture tube had been washed on several suc-

cessive days to remove the nutrient media and left standing for several weeks on the surface of moist filter paper, much rotted willow wood in water, or in thin films of water in Petri dishes. Conidia, and occasionally mycelial fragments, washed from such extremely depleted cultures and left standing deeply submerged in water germinated and many gave rise to sporangia. Conidia washed from the same original cultures and left standing in water during the same period or even much longer never germinated. The long exposure to the air seemingly made such germinations possible.

Feeling that development of sporangia might be definitely traceable to an oxygen requirement mycelium was placed in water and in nutrient media in flasks and connected in series by means of Y-tubes with an aspirator so that air was constantly bubbling through the cultures. In no case did sporangia develop abundantly.

As a result of many trials the following general conclusions as to the conditions essential to the development of sporangia seem evident:

1. That a great reduction in the available nutrition is essential.
2. That sporangia develop only on hyphae that are at or near the surface of the water or from germinating spores that have had long exposure to the air.
3. That alternate drying, to a point as dry as can be attained without injury to the hyphae, and wetting are favorable.

Thus it seems that *A. rubescens* has become so adapted to the fluctuating conditions that must prevail in a slime flux that it must have similar conditions to stimulate it to development of sporangia.

The reactions of the fungus to air are very striking. Very little growth takes place under a cover glass. Stray hyphae near the edge of the cover glass will send out many branches toward the edge of the cover, which will branch and rebranch until a perfect tangle of hyphae is formed just beneath the edge of the cover. Where the hyphae are smaller and copiously branched conidia usually develop abundantly beyond the cover glass; where the hyphae are larger and less branched sporangia develop. A characteristic portion of a sporangium-bearing growth at the

edge of a cover glass is shown in figure 35. The heavy line upon which the letters are placed represents the edge of the cover glass. In this case the distance between A–B and G–H was slightly shortened. Otherwise all actively growing hyphae are shown in position. The sporangium-bearing hyphae all grew from a mass of mycelium under the cover but near its edge. The insets show development that had taken place two days later. Spores were matured and discharged normally. No attempt was made to show spore masses, as repeated waterings and handling of the slide moved them from the places where they would normally have been found. Sporangia are stippled to make them more easily distinguishable but no attempt has been made to show cell contents.

Figures 24–27 and 35 are arranged to show the variations in sporangial arrangement ordinarily observed in such cultures. A sporangium-bearing hypha is first definitely recognizable as a branch which apically increases its diameter as it develops. Soon the end cell enlarges and the protoplasm becomes more dense (FIG. 35, *D*, *M*). Here, as in materials taken from the slime flux about 3 days is required for the development and discharge of a sporangium. After discharge a proliferating hypha grows into it which may form a cross wall at once and form a sporangium (as shown on the branch to the left, figure 25) or develop several cells before forming a second sporangium (figure 25, main hypha, 27, or most cases shown in figure 35). Here also sessile and lateral sporangia often occur (FIG. 35, *G*, *P*). It is not uncommon to find two proliferations ramifying the same sporangium (FIGS. 24, 35, *O*). If a terminal sporangium failed to discharge normally its spores, a sympodial development was observed (FIG. 35, *A*). Figure 35 shows discharged sporangia under the edge of the cover glass but none were ever observed forming or maturing there. The position of the cover glass had evidently been slightly changed by handling. All such mounts showed more or less contamination with bacteria, yeasts, and other fungi because of their repeated exposures to the air.

MECHANISM OF DISCHARGE

Literature on *Ascoidea* abounds with discussions as to how the spores get out of the sporangium. The general conclusion reached in each case, however, was that the onward growth of the proliferating hypha was largely responsible for the discharge. Brefeld (1), however, observed that the intersporal slime tended to swell and that the inner layers of the wall of the sporangium gelatinized, and he suggested that these might assist in initiating the discharge but that eventually the spores were pushed out. Surely their materials were much less vigorous than the ones used in these studies or they actually failed to observe normal discharges. The discharges illustrated by Brefeld (1) resemble the ones shown in figure 34, an abnormality that was several times observed. In such cases the spores would have to be pushed out if they ever escaped. No case was ever observed where spores remaining in a sporangium were pushed out by a proliferating growth. If much of the spore mass was left in the sporangium usually no proliferation occurred. If only a few spores remained they were pushed aside instead of out during proliferation. The discharges illustrated in figures 1, 2, 4 are characteristic of all observed. These figures (as 1*d*, 2*d*, 3, etc.) all show that the spores had been entirely discharged before proliferation occurred. Normally there is little if any outward bulging of the intervening cross wall before the discharge. Where the cross wall does bulge (FIGS. 1*d*, 4*a*) it might function as a columella in initiating a discharge. It seems more probable that the discharge is largely brought about by the accumulated osmotic pressure due to the transformation of glycogen, abundantly present in the young sporangium, and absent in the mature sporangium, into compounds [6] with high osmotic pressure held in the intersporal slime. That the intersporal slime has an enormous power for water absorption is shown by its great expansion after discharge and by the appearance of many vacuoles (FIG. 1). The release of tension upon the rupturing of the sporangium wall is sufficient to force out the greater part of the spore mass and the remainder may be drawn out by the tenacity of the intersporal slime.

[6] Microchemical tests failed to indicate their identity.

The innermost layer of the wall of the sporangium also appears to aid in the discharge. Discharges observed in slide cultures show a clear, apparently gelatinous, expansion of the innermost layer of the wall at the time of the discharge which so nearly fills the lumen of the empty sporangium that only a narrow channel remains through the center of the sporangium. This was much more conspicuous in sporangia which discharged abnormally and where some of the content of the sporangium remained in the central channel as shown in figures 26, 29, 32, and 58. In completely emptied sporangia this channel is only visible when the light is especially carefully adjusted as both are perfectly transparent. This modification of the inner wall was not observed in materials taken from the slime flux but the granules covering the outside of the sporangium in such materials would have made its observation very difficult, if not impossible.

The above observations point to the conclusion that the discharge is primarily brought about by high osmotic pressure developed by substances in the intersporal slime. It is assisted by the gelatinization of the inner layer of the wall of the sporangium and often at least by the slight bulging of the septum from pressure below.

IDENTITY OF THE AMERICAN ASCOIDEA

As previously stated, only two species of *Ascoidea* have been described: *A. rubescens* by Brefeld and Lindau (**1**), and *A. saprolegnioides* by Holtermann (**2**). Holtermann distinguished his species on two points of morphological difference: that several vegetative cells were formed following a proliferation before another sporangium and that the sporangiospores lacked the hat-shape. Brefeld both describes and figures (FIGS. 18, 19) cases where vegetative cells were interspersed between sporangia on proliferating hyphae and Holtermann, cases where no vegetative cells intervened (FIGS. 19, 21). This appears to be at best a fluctuating characteristic and should hardly be considered a valid reason for separating two species. Thus it seems that the only possible point of separation of the two species is the absence of hat-shaped spores in *A. saprolegnioides*.[7] The American

[7] Holtermann's figures suggest that his lenses may not have been adequate for observing delicate details. The "brims" characteristic of hat-shaped

Ascoidea agrees very closely with Holtermann's description of his fungus except that it has hat-shaped spores. Since the hat-shaped spores are the most definite point for separating the two species it seems that the American form must be *A. rubescens* and that in this fungus extremes of variation in sporangial development may occur. All structures described by Brefeld have been observed in the American form, except a few abnormalities, and the occurrence of sporangiospores in twos which was denied by Popta (**4**) working with the same materials. *A. rubescens* is described as having a fishy smell which has not been observed in the American *Ascoidea*.

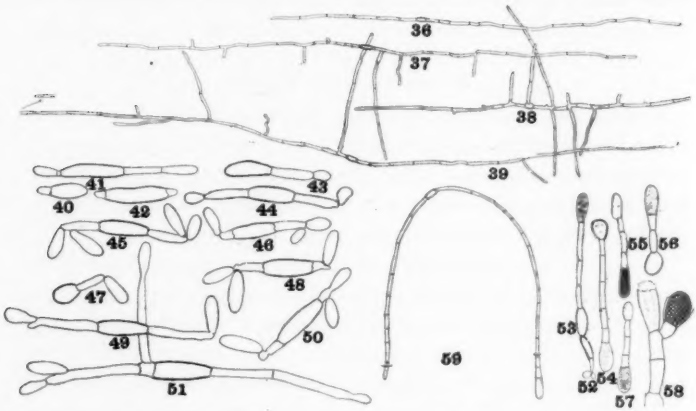
SPORE GERMINATIONS

One of the very striking characteristics of the spores, both sporangiospores and conidia, is that they rarely if ever germinate in cultures or in the slime flux where they are formed but germinate readily when spread out in a thin film of water or nutrient media, or on the surface of agar media.

Conidia.—The germination of conidia has been well described by Brefeld (**1**). In the author's studies extremes of variations were observed under varying conditions. Three extreme types with intergradations were observed. First, figures 36–39 show four conidia germinating on the surface of nutrient agar after 2 days. Under such conditions a branching septate mycelium develops at once. It was from such germinations that stock cultures were secured. Second, if conidia are placed in thin films of water the germ tubes at once proceed to form conidia (FIGS. 40–51). The details of conidial formation show well here and are identical with conidial formation on vegetative hyphae. Such germinations were always observed around the edges of cover glasses where mounts were kept in moist chambers to secure sporangia, or where mycelia or water containing spores were spread out in thin films in Petri dishes or on slides. Elongated, oval, and thick-walled conidia all germinate alike except that the oval, or smaller, conidia usually develop only one germ tube

spores are so transparent that they are not easily visible. It seems quite possible that if Holtermann's materials had had the hat-shape the fact might readily have been overlooked. Since this is the only definite distinguishing characteristic the validity of his species seems questionable.

(FIGS. 47, 52). A third extreme type of germination is illustrated in figures 53–57. In this case old mycelia from nutrient media had been placed on wet filter paper in a Petri dish in order that the possible development of sporangia might be watched more carefully. Many conidia and some sporangia developed. When the culture became depleted the spores were washed with water into a Petri dish and left standing. Several days later when examining the dish it was found that the conidia had germinated very abundantly. Some had germinated by directly forming



FIGS. 36–59. Germinations of conidia. 36–39. Four conidia germinating on agar medium, 2d at 23° C. 40–51. Germinations of conidia in thin films of liquid, 40–42 after 7 h, 43–51 after 24 h. 52–57. Germination of conidia from old depleted culture; 52, forming conidia and others forming sporangia. 58. Sporangia developing from a mycelial fragment. 59. A conidium germinating under edge of cover-glass (dotted line) and forming a sporangium beyond the cover-glass. 36–39 × 34; 40–51 × 188; 53–58 × 100; 59 × 44.

conidia (FIG. 52), others only germ tubes, and still others, about half, had formed sporangia on the ends of very short germ tubes (FIGS. 53–57). A few of the sporangia had discharged spores. Where fragments of hyphae occurred many of them had also proceeded to develop sporangia (FIG. 58). This type of germination was often observed under similar conditions. Intermediate between these extremes every gradation was observed. A short mycelium might develop and then proceed to form conidia, or

sporangia. Figure 59 shows a germinating conidium that lay just under the edge of the cover glass. Where submerged under the cover glass a tube resulted but when it reached the edge of the cover, as shown by dotted lines, sporangia developed. Conidial development was even more common in such cases. Very often under such conditions the hyphae branched profusely before reaching the edge of the cover glass and as the branches intermingled a mat of radiating hyphae, all having developed from spores lying just under the cover, is formed.

In many larger conidia, before germination, the protoplasm may appear as if endogenous spores were being formed, as in sporangia, but no endogenous spores matured. Similar observations were recorded by Brefeld (1).

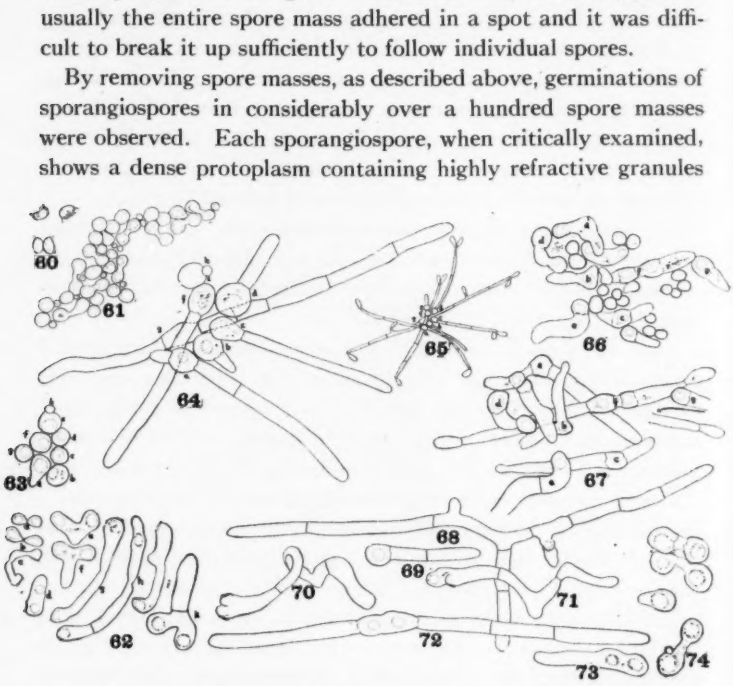
That an abundance of air is a prime necessity for conidial germinations was quite clearly shown by aspirating cultures. In unaërated cultures, the growth was confined to the original inoculum but in aërated cultures tiny colonies, evidently coming from germinating conidia, almost filled the nutrient media used.

Sporangiospores.—Extreme difficulty was experienced in studying the germination of the sporangiospores. While a few sporangia were formed in pure cultures it was almost impossible to find the discharged spore masses. The necessity of exposing cultures to the air to secure abundant sporangia almost always resulted in contaminations of various sorts. In spite of the fact that many attempts were made, using various methods, sporangia, without contamination, were rarely, if ever, secured. Even where seemingly clean spore masses were obtained the necessity for exposure to the air to secure germination of the spores again caused difficulty.

The removal of spore masses from the culture drops in which they had been discharged was very simple. Being held together by the oily slime, the entire spore mass, unless caught by hyphae, came to the surface of the liquid in which it was discharged. It was then relatively easy to reach with a needle, while watching under low power, and remove the entire spore mass as it would adhere to the needle. Some difficulty was experienced in removing the spore mass from the needle. It was almost impossible to free the spore mass from the needle in water but it could be

quite successfully removed by rotating the needle on the surface of a dry slide or cover glass. The slime was so tenacious that usually the entire spore mass adhered in a spot and it was difficult to break it up sufficiently to follow individual spores.

By removing spore masses, as described above, germinations of sporangiospores in considerably over a hundred spore masses were observed. Each sporangiospore, when critically examined, shows a dense protoplasm containing highly refractive granules



FIGS. 60–74. Germination of sporangiospores. 60. Detail of sporangiospores. 61. Ungerminated (small) and germinating (larger) sporangiospores at edge of a spore-mass in water. Many are fusing in pairs. 62. Types of anastomoses commonly observed in nutrient media after 2–4 days. 63–65. Development in an isolated group of sporangiospores in nutrient solution where no fusions occurred, 63, after 2 days, 64, 3 days, and 65, 4 days. 66, 67. Development in an isolated group of sporangiospores in nutrient solution showing the most commonly observed types of germinations, 66, after 4 days and 67, 5 days. 68, 70, 71. Sporangiospore germinations on nutrient agar after 3 days and 69, 70, 73, after 4 days in nutrient fluid. 74. Detail of anastomoses. Figs. 60, 63, 64, 74 × 286; fig. 65 × 50; other figs. × 215.

and a large central vacuole (FIG. 60). Soon after sporangiospores are placed in a thin film of water they swell enormously and protrude germ tubes which usually fuse in pairs but rarely does germination progress beyond this point. Figure 61 shows

the appearance at the edge of a spore mass where the spores were sufficiently separated to be seen clearly. The small rounded bodies are ungerminated spores, the larger rounded cells, spores that have enlarged but not yet developed tubes, and the others the spores whose germ tubes have fused. During the germination the central vacuole of the spore becomes greatly enlarged. The vacuoles are indicated by dotted lines in the spores. Early stages in this fusion appear as shown in figure 62*a* and *b*, and a little later stages, more highly magnified as seen in figure 74. In such cases it is usually very difficult to observe the "brim of the hat." The fusion of tubes does not always occur between spores lying in close proximity to each other. A tube may pass by a number of spores and fuse with the tube of another at a distance. In such cases fusions appearing as shown in figure 62*c*, *g*, *h*, and *i* are common. In water usually the germinations proceed no further but occasionally short hyphae arise from the fused germ tubes as shown in figure 62*d*, *e*, *f*, *i*, and *k*. If, however, a nutrient solution was added the fused germ tubes gave rise to hyphae on which conidia develop promptly.

If, however, the spore masses are placed in a nutrient solution fusions of spores are less common. Figures 66 and 67 show such a mass; figure 66 shows the mass 4 days after the drop was made and 67 a day later. As it was a small mass of isolated spores it was hoped that it might be possible to follow individual spores but the growth of the germ tubes disarranged the spores so greatly that it was impossible to follow many of the spores. Only those that could be quite positively identified are shown in figure 67. Other spores, germinated in a nutrient solution (beef extract + dextrose + elm decoction), are shown in figures 69, 72, and 73. It may be seen that in some cases hyphae arise from fused spores (FIGS. 66, 67*a*, *f*, 72–73) and in other cases from individual spores (FIGS. 67*b*, *c*, *e*, *g*, 69). Regardless of their origin the resulting hyphae at once proceed to form conidia exactly as did tubes arising from conidia.

Figure 63 shows another isolated group of 8 sporangiospores after 2 days in a nutrient solution. One spore "*h*" did not swell. The others have all enlarged more or less and "*a*" has started to develop a germ tube. In figure 64 the development during the

next 24 hours is shown. All of the enlarged spores but "*e*" have developed germ tubes. A day later figure 65, "*e*," had developed a germ tube and all of the others were forming conidia. This type of germination was relatively uncommon under the conditions used. (In one series 2 out of 30 spores gave rise directly to germ tubes, in two series most germinated in this fashion and in 12 series about half fused and the other half gave rise directly to germ tubes. In the remaining series hyphae seemed to arise in most cases from fused spores but in many cases it was impossible to be positive because of the crowded condition of the spores.) The variations in behavior noted may have been due to changes in the nutritive medium. Where mounts are held in a saturated atmosphere in moist chambers the moisture collecting on the slide and even dripping from the moist chamber to the slide at times would greatly vary the concentration of the liquid nutrient medium used.

When sporangiospores were planted on the surface of nutrient agar a wide spreading mycelium resulted exactly as when conidia were planted on agar. In all cases bacteria restricted the growth. Figures 68, 70, and 71 show germinating spores that were removed from the surface of agar (beef extract + dextrose + elm decoction) for examination under the microscope after 3 days' growth. In all three cases the hyphae seem to have come from fused germ tubes but it was impossible to be sure.

During germination it was often impossible to distinguish the "brims" of the hat-shaped spores but when observed the germ tubes usually seemed to emerge parallel with them (FIG. 62*i*, *d*, *c* ABOVE) while less often it emerged opposite the "brim" (FIG. 62*c* BELOW). While the "brims" were very difficult to see in unstained materials they show well in stained mounts, over a hundred of which have been examined.

The fusions of the germ tubes of sporangiospores resemble those well known for the ascospores of *Saccharomycodes Ludwigii*, *Willia saturnus* and the yeast of Johannisberg II but the resemblance may be entirely superficial. No evidence has been secured to indicate that the fusion of germ tubes has a sexual significance.

During the process of germination the slime surrounding the

spores gradually disappears. Brefeld (1) observed this and concluded that it was used by the germinating spores. This is probably true but it does not seem to contain sufficient nutrition for their complete germination as in no case were well developed tubes secured unless additional nutrition was available. Brefeld's description of the germination of the sporangiospores quite markedly differs from that just described in certain details. He figures and describes the spores as rupturing in 2 halves much as is characteristic of the ascospores of *Aspergillus* and did not observe fusions of spores on germination. He, however, described the development of conidia from germinating sporangiospores. Holtermann (**2**) found that long, septate threads arise from the sporangiospores which gave rise neither to sporangia nor conidia. These are variations which might readily occur under varied cultural conditions.

CONCLUSIONS

1. An *Ascoidea*, held to be *A. rubescens*, was collected in slime fluxes at Ithaca, N. Y., and at Lincoln, Nebr. These findings apparently are the first records of the fungus in North America.

2. The development of sporangia and conidia, both from the slime flux and in cultures, has been studied.

3. Sporangia only develop on hyphae near the surface of water. A great reduction in the available nutrition is essential and alternate drying and wetting are favorable conditions.

4. Oval and cylindrical conidia develop either in the air or, if on submerged hyphae, near the surface. Thick-walled, resting conidia develop on submerged hyphae.

5. Exposure on the surface of agar or in thin films of liquid is essential for the germination of both conidia and sporangiospores.

6. Both types of spores upon germination give rise at once to conidia when nutrition is low and to well developed hyphae under conditions of higher nutrition. When very depleted conidia may almost directly give rise to sporangia upon germination.

7. Sporangiospores commonly fuse upon germination.

The writer wishes to express her thanks to Prof. H. M. Fitzpatrick, of Cornell University, for his stimulating interest in these

studies and for suggestions made after reading a draft of this paper to Prof. E. N. Andersen for assistance in making microchemical tests, and to Prof. T. J. Fitzpatrick, of the University of Nebraska, for editing and proof reading the paper.

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NOTES ON SOME RUST COLLECTIONS FROM COLORADO, WYOMING, AND SOUTH DAKOTA [1]

H. W. Thurston, Jr., and F. D. Kern

During the summer of 1929 Messrs. Fred J. Seaver and Paul F. Shope made some interesting collections of rusts in Colorado,[2] and, together with Mr. Junius Henderson, some further collections in South Dakota.

Their collections, together with several others which were made in Wyoming by the mycologists in attendance at the summer meeting of the Botanical Society of America, have recently been examined by the writers. In the list which follows, the collectors are not specifically named but should be understood to be as here indicated.

While the rusts of Colorado have become fairly well known, as the result of the work of such collectors as Arthur, Kern, Bethel, Gooding et al., the rust flora of Wyoming and South Dakota is not nearly so well known.

In determining these collections, several new localities were recorded for certain rust species as well as several hosts which are new, either for North America or for the region. In view of the fact that the volume on the Rusts of North America (Vol. 7 of the No. Am. Flora) is no longer open for the recording of additions and corrections in the distribution of rust species and their hosts it has seemed desirable to present a list of these recent collections. The list contains a total of ninety-seven collections, representing twelve genera and fifty-three species of rusts. Eleven host plants are here recorded as new for North America, one as new for Colorado, nine as new for Wyoming and seven as new for South Dakota, while six of the rust species are here reported from their respective localities for the first time. The three collections are numbered as one series; the numbers in this

[1] Contribution from the Department of Botany, the Pennsylvania State College, No. 73.

[2] See Mycologia **22**: 1–8, 1930.

list are those as they appear on the specimens in the Herbaria of the New York Botanical Garden, the University of Colorado, and the Pennsylvania State College. The hosts were determined by P. A. Rydberg with the exception of those in the genus *Carex*, which were determined by K. K. MacKenzie.

CALYPTOSPORA COLUMNARIS (Alb. & Schw.) Kuhn on *Vaccinium* sp., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *199*.

COLEOSPORIUM RIBICOLA (Cooke & Ellis) Arth. on *Ribes inebrians* Lind., Gregory Canyon, Colo., Aug. 13, *349*.

CRONARTIUM HARKNESSII (J. P. Moore) Meinecke on *Castilleja sulphurea* Rydb., Sylvan Lake, So. Dak., Aug. 8, *300*. First report for South Dakota.

GYMNOSPORANGIUM BETHELI Kern on *Crataegus succulenta* Shrad., Gregory Canyon, Colo., Aug. 13, *348*. New host for North America.

MELAMPSORA BIGELOWII Thüm. on *Salix* sp., Univ. of Colorado, Colo., July 29–30, *94*.

MELAMPSORA HUMBOLDTIANA Speg. on *Salix amygdaloides* Anderson, Edgemont, So. Dak., Aug. 6, *272*. Wind Cave, So. Dak., Aug. 7, *277*. New host for North America.

MELAMPSOROPSIS PYROLAE (DC.) Arth. on *Pyrola minor* L., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *166*. New host for Wyoming; the only previous report on this host is from Greenland.

POLYTHELIS THALICTRI (Chev.) Arth. on *Thalictrum dasycarpum* Fisch. & Lall., Wind Cave, So. Dak., Aug. 9, *329*. Not before reported from South Dakota.

PHRAGMIDIUM IVESIAE Sydow on *Potentilla glaucophylla* Lehm., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *208*, *221*. New host for Wyoming.

PHRAGMIDIUM MONTIVAGUM Arth. on *Rosa* sp., Univ. of Colorado Camp, Colo., July 29–30, *124*. Gregory Canyon, Colo., Aug. 13, *362*.

On *Rosa suffulta* Greene, Wind Cave, So. Dak., Aug. 7, *274*. First report on this host for North America.

PHRAGMIDIUM PECKIANUM Arth. on *Oreobatus deliciosus* (Torr.) Rydb., Middle Boulder Canyon, Colo., July 23, *1*.

PHRAGMIDIUM SPECIOSUM (Fries) Cooke on *Rosa* sp., Gregory Canyon, Colo., Aug. 13, *365*.

PUCCINIASTRUM AGRIMONIAE (Schw.) Tranz. on *Agrimonia striata* Michx., Legion Camp, So. Dak., Aug. 8, *316*. New host for North America.

PUCCINIA ABSINTHII (Hedw. f.) DC. on *Artemisia cana* Pursh., Antelope, So. Dak., Aug. 6, *269*, *270*, near Edgemont, So. Dak., Aug. 10, *337*. This is a new host for South Dakota.

On *Artemisia cuneata* Rydb., Pikes Nat. Forest, Colo., Aug. 14, *378*. New host for North America.

PUCCINIA AEMULANS Sydow on *Gymnolomia multiflora* (Nutt.) B. & H., Pikes Nat. Forest, Colo., Aug. 16, *385*.

PUCCINIA ASTERUM (Schw.) Kern on *Aster* sp., Middle Boulder Canyon, Colo., July 26, *72*. Univ. of Wyoming Camp, Wyo., Aug. 1–4, *214*.

PUCCINIA CIRSII Lasch. on *Cirsium megacephalum* (A. Gray) Cockerell, Gregory Canyon, Colo., Aug. 13, *353*.

On *Cirsium* sp., Univ. of Colorado Camp, Colo., July 29–30, *121*.

PUCCINIA CLEMATIDIS (DC.) Lagerh. on *Thalictrum* sp., Univ. of Colo. Camp, Colo., July 29, *123*.

On *Bromus ciliatus* L., Wind Cave, So. Dak., Aug. 9, *330*. This is a new host for South Dakota.

PUCCINIA CRUCIFERARUM Rudolphi on *Cardamine cordifolia* A. Gray, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *161*.

PUCCINIA GENTIANAE (Strauss) Link on *Dasystephana* sp., Gregory Canyon, Colo., Aug. 13, *360*.

PUCCINIA GRINDELIAE Peck on *Grindelia texana* Scheele, Gregory Canyon, Colo., Aug. 13, *363*.

PUCCINIA GROSSULARIAE (Schum.) Lagerh. on *Ribes americanum* Mill., Wind Cave, So. Dak., Aug. 9, *331*.

PUCCINIA GRUMOSA Syd. & Holw. on *Anticlea coloradensis* Rydb., Middle Boulder Canyon, Colo., July 25, *70*. First report for Colorado.

PUCCINIA HEMISPHAERICA (Peck) E. & E. on *Lactuca pulchella* DC., Edgemont, So. Dak., Aug. 10, *339*.

PUCCINIA HELIANTHI-MOLLIS Schw. on *Helianthus lenticularis* Dougl., Univ. of Wyoming Camp, Wyo., Aug. 5, *265*. New host for Wyoming.

On *Helianthus fascicularis* Greene, Gregory Canyon, Colo., Aug. 13, *370*.

PUCCINIA HEUCHERAE (Schw.) Dietel on *Mitella* sp., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *256*. This is a new locality for this rust. The specimen is referred to *P. Heucherae* although it differs somewhat since the spores are nearly colorless.

PUCCINIA HIERACIATA (Schw.) H. S. Jackson on *Agoseris parviflora* (Nutt.) Dietr., Univ. of Colorado Camp, Colo., July 29, *133*.

On *Agoseris humilis* Rydb., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *173*. This is a new host for North America.

On *Agoseris scorzoneraefolia* (Schrad.) Greene, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *211*. This host is also new for North America.

On *Agoseris aurantiaca* (Hook.) Greene, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *259*. New host for Wyoming.

On *Agoseris* sp., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *174*, *261–II*.

On *Carex siccata* Dewey, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *212*. New host for Wyoming.

PUCCINIA HIERACII (Schum.) H. Mart. on *Taraxacum* sp., Univ. of Colorado Camp, Colo., July 29, *112*.

On *Crepis* sp., Univ. of Colorado Camp, Colo., July 29, *122*.

On *Taraxacum Taraxacum* Karst, Gregory Canyon, Colo., Aug. 13, *364*.

On *Crepis runcinata* (James) T. & G., Wind Cave, So. Dak., Aug. 9, *296*. New host for South Dakota.

On *Hieracium scabriusculum* Schw., Wind Cave, So. Dak., Aug. 7, *278*. New host for United States.

PUCCINIA INTERMIXTA Peck on *Iva axillaris* Pursh, Edgemont, So. Dak., Aug. 6, *271*. Edgemont, So. Dak., Aug. 10, *338*.

PUCCINIA LIGUSTICI Ellis & Ev. on *Angellica Grayi* C. & R., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *224*. New host for Wyoming.

On *Ligusticum Porteri* Cooke & Rav., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *216*. New host for Wyoming.

On *Ligusticum simulans* Cooke & Rav., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *169*. New host for North America.

On *Oxypolis Fendleri* (Gray) Heller, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *171*, *176*. New host for Wyoming.

PUCCINIA MALVACEARUM Bertero on *Malva pusilla* Sm., Gregory Canyon, Colo., Aug. 12, *347*. New host for North America.

On *Althea rosea* Cav., Gregory Canyon, Colo., Aug. 13, *355*.

PUCCINIA MENTHAE Pers. on *Mentha Penardi* (Briq.) Rydb., Gregory Canyon, Colo., Aug. 13, *357*. Also from Coal Creek Canyon, Colo., specimen not numbered.

On *Monarda Ramaleyi* A. Nels., Wind Cave, So. Dak., Aug. 7, *273*. New host for South Dakota.

PUCCINIA POLYGONI-VIVIPARI H. Dietr. on *Bistorta vivipara* (L.) S. F. Gray, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *175*, *220*.

On *Oxypolis Fendleri* (Gray) Heller, I, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *178*. New host for North America.

PUCCINIA POCULIFORMIS (Jacq.) Wettst. on *Hordeum jubatum* L., Edgemont, So. Dak., Aug. 10, 340. Gregory Canyon, Colo., Aug. 13, *372*.

On *Phleum pratense* L., Gregory Canyon, Colo., Aug. 13, *358*.

PUCCINIA SCABER (Ellis & Ev.) Barth. on *Stipa Lettermanii* Vasey, Pikes Nat. Forest, Colo., Aug. 16, *384*.

PUCCINIA SCANDICA Johans. on *Epilobium anagallidifolium* Lam., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *177*. New locality for this species, also new host for North America. The specimen bears also a few aecia of what appear to be *Puccinia Peckii* (Detoni) Kellerm.

PUCCINIA SHERARDIANA Körn. on *Sphaeralcea coccinea* (Nutt.) Rydb., Gregory Canyon, Colo., Aug. 13, *368*.

PUCCINIA TRELEASIANA Paz. on *Caltha rotundifolia* (Huth.) Greene, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *240*. Middle Boulder Canyon, Colo., July 24, *38*.

PUCCINIA URTICATA (Link) Kern on *Carex nebraskensis* Dewey, Coal Creek Canyon, Colo., Aug. 23, *513*.

PUCCINIA VIOLAE (Schum.) DC. on *Viola adunca* Sm., Wind Cave, So. Dak., Aug. 7, *279*. New host for South Dakota.

PUCCINIA XANTHII Schw. on *Xanthium speciosum* Kearney, Antelope, So. Dak., Aug. 6, *268*.

UROPYXIS AMORPHAE (M. A. Curt.) Schröt. on *Amorpha canescens* Pursh., Wind Cave, So. Dak., Aug. 7, *280*, Aug. 9, *334*.

UROPYXIS SANGUINEA (Peck) Arth. on *Odostemon aquifolium* (Pursh) Rydb., Gregory Canyon, Colo., Aug. 13, *352*.

UROMYCES AEMULUS Arth. on *Allium brevistylum* S. Wats., Univ. of Wyoming Camp, Wyo., Aug. 1–4, *162*.

UROMYCES ARGOPHYLLAE Seym. on *Psoralea argophylla* Pursh., Wind Cave, So. Dak., Aug. 7, *298*, Aug. 9, *336*.

UROMYCES FABAE (Pers.) DeBary on *Lathyrus* sp., Pikes Nat. Forest, Colo., Aug. 14, *379*.

UROMYCES GLYCYRRHIZAE (Rab.) Magn. on *Glycyrrhiza lepidota* Nutt., Wind Cave, So. Dak., Aug. 9, *335*.

UROMYCES HETERODERMUS Sydow on *Erythronium parviflorum* (S. Wats.) Gooding, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *163*.

UROMYCES JONESII Peck on *Ranunculus alismellus* Geyer, Middle Boulder Canyon, Colo., July 26, *65*. This is a new host for Colorado. Univ. of Wyoming Camp, Wyo., Aug. 1–4, *165*, *209*, *258*. First report of this rust from Wyoming.

UROMYCES OBLONGUS Vize on *Trifolium Parryi* A. Gray, I, Univ. of Wyoming Camp, Wyo., Aug. 1–4, *219*, *237*. New host for Wyoming.

On *Trifolium* sp., III, Univ. of Wyoming Camp, Wyo., Aug. 1–3, *223*.

UROMYCES SILPHII (Burrill) Arth. on *Juncus Dudleyi* Wiegand' Gregory Canyon, Colo., Aug. 13, *359*.

UROMYCES SUBSTRIATUS Sydow on *Lupinus argenteus* Pursh, Gregory Canyon, Colo., Aug. 13, *351*.

UROMYCES TRIFOLII (Hedw. f.) Lév. on *Trifolium repens* L., Wind Cave, So. Dak., Aug. 8, *319*. New host for South Dakota.

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A RARE PHALLOID FROM THE NEW YORK BOTANICAL GARDEN

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(WITH PLATE 8)

In 1916, David R. Sumstine reported a new species of *Colus* (Mycologia **8**: 183. 1916) from Pennsylvania under the name *Colus Schellenbergiae*. The material from which this species was described was collected in the yard of Mrs. F. F. Schellenberg of Pittsburgh, Pennsylvania. The author of the species calls attention to the fact that the description of *Colus javanicus* Penzig agrees in a general way with his plants but states that he had no opportunity to compare the specimens.

In 1928, the writer discovered in an obscure corner of The New York Botanical Garden a group of phalloids which seem to agree in every way with the description given by Sumstine for his Pittsburgh material. This was collected throughout the entire summer, several of the plants appearing after each rain storm, the eggs remaining dormant during the intervening dry periods. Numerous collections were obtained and colored illustrations were made from the living material by Miss Mary E. Eaton. A report of this species was made before the Torrey Botanical Club at The New York Botanical Garden and a brief note published in connection with the minutes of that meeting (Torreya **29**: 49. 1929). This has apparently been overlooked by mycologists, for no one has since mentioned the occurrence of this species outside of the Pittsburgh region.

Each season since the discovery of this plant in The New York Botanical Garden, it has continued to appear over an area scarcely more than twenty feet in diameter. Just why it should appear there and in no other place or how it got there are questions which it is impossible to answer. Since the above observations were made the writer has secured a small specimen of *Colus javanicus* from the Director of the Botanic Garden at Buitenzorg, Java. So far as it is possible to judge from preserved material

the American and Javan species are identical, as intimated by Sumstine in his original description.

During the winter of 1929, the writer received from Brother Leon of Cuba a specimen preserved in glycerine which also appears to be identical with the American and Javan specimens. This gives a very interesting distribution, assuming that the three are identical.

C. G. Lloyd has established a genus *Pseudocolus* to include those species of *Colus* in which the arms arise from a stem. This distinction would not appear to be a good one since in the young specimens the stem is not apparent, but appears as the specimens mature. The following is the synonymy of the species:

COLUS SCHELLENBERGIAE Sumstine, Mycologia **8**: 183. 1916.

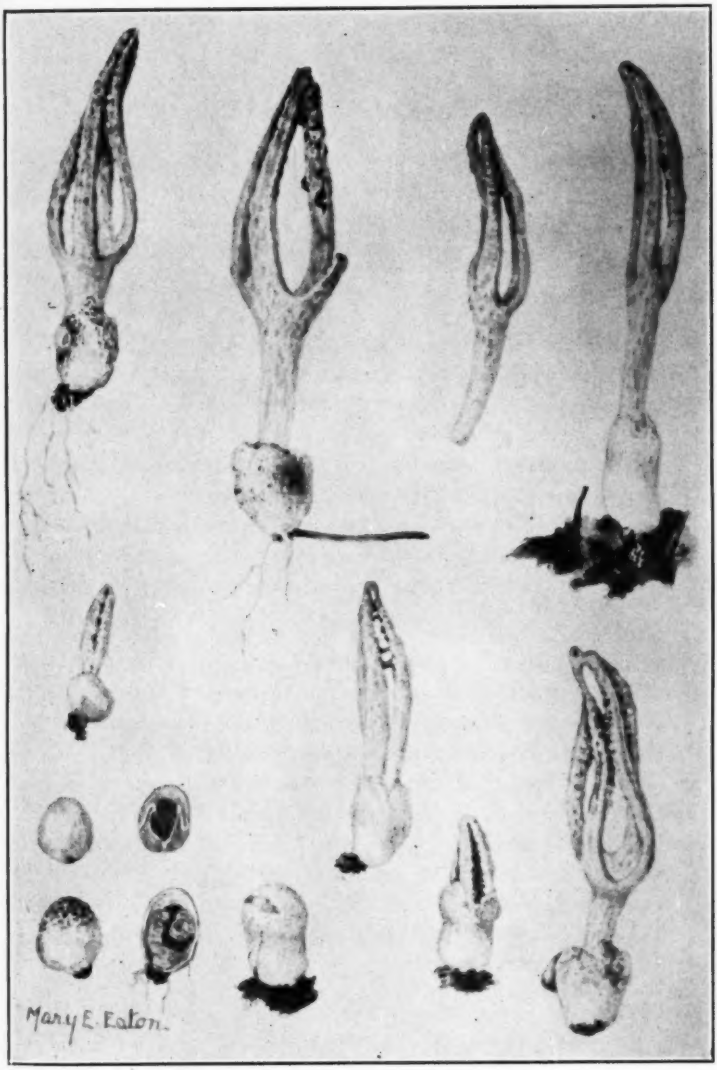
?Colus javanicus Penzig, Ann. Jard. Bot. Buitenzorg II. **1**: 160. 1899.

Pseudocolus Schellenbergiae Johnson, Ohio Biol. Survey Bull. **22**: 338. 1929.

THE NEW YORK BOTANICAL GARDEN,
BRONX PARK, NEW YORK

EXPLANATION OF PLATE 8

Photograph of the various stages in the development of *Colus Schellenbergiae* as it appeared in The New York Botanical Garden. The photographs were made from the colored drawings by Miss Mary E. Eaton and are about natural size.



COLUS SCHELLENBERGIAE

NOTES AND BRIEF ARTICLES

While MYCOLOGIA is not usually sold separately, anticipating a demand for the present issue, which has been reproduced at rather heavy cost, extras have been printed and may be had through The New York Botanical Garden at one dollar ($1.00) each.

Doctor David W. Fairchild, long in charge of the office in the Bureau of Plant Industry dealing with plant introductions, visited the Garden Friday, October 31. Mycologists will be glad to learn that he is still interested in *Basidiobolus*, a fungus on the cytology of which he made an important contribution many years ago. Now with every opportunity and condition favorable to research may we not hope to see Dr. Fairchild endeavor to clear up some of the puzzling questions still being raised regarding the nature of sex and reproduction in such fungi.

Dr. Alfred H. Povah is spending this year at the Cryptogamic Laboratories of Harvard University assisting Professor William H. Weston, Jr., and in the Farlow Herbarium studying the fungi collected last summer by the Botanical Party of the Michigan State Survey of Isle Royale, Lake Superior, under the auspices of the University of Michigan Herbarium. Approximately 2100 collections of fungi and lichens were obtained by the party consisting of Dr. Alfred H. Povah, in charge of Cryptogams, assisted by Mr. J. L. Lowe, Jr., and Mr. Clair A. Brown, in charge of Phanerogams, assisted by Mr. J. B. McFarlin.

The New York Botanical Garden has recently received a valuable addition to its collection of cultures of fungi from Dr. Charles Thom of the Bureau of Chemistry and Soils, Department of Agriculture, Washington, D. C. This gift, consisting of something over two hundred cultures of species of *Aspergillus*

and *Penicillium*, is especially valuable at this time, since the Garden is cooperating with the Laboratory of Mycology, Department of Dermatology, of the College of Physicians and Surgeons of Columbia University, and it is known that several species of the fungi in these groups have been found associated with certain cases of asthma and other human diseases. The set is one of five being distributed to central laboratories in the United States. We are certainly very grateful to Dr. Thom for his contribution.